PFOS:
A FROG EMBRYO TERATOGENESIS ASSAY – *XENOPUS* (FETAX)

FINAL REPORT

WILDLIFE INTERNATIONAL, LTD. PROJECT NUMBER: 454A-116

3M LAB REQUEST NO. U2723

ASTM Method E 1439-91

AUTHORS:

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STUDY INITIATION DATE: May 11, 2000

STUDY COMPLETION DATE: April 6, 2001

SUBMITTED TO:

3M Corporation
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935 Bush Avenue
St. Paul, Minnesota 55144

Wildlife International, Ltd.
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GOOD LABORATORY PRACTICE COMPLIANCE STATEMENT

SPONSOR: 3M Corporation

TITLE: PFOS: A Frog Embryo Teratogenesis Assay – *Xenopus* (FETAX)

WILDLIFE INTERNATIONAL, LTD. PROJECT NUMBER: 454A-116

STUDY COMPLETION: April 6, 2001

This study was not conducted in compliance with Good Laboratory Practice Standards as published by the U.S. Environmental Protection Agency in 40 CFR Parts 160 and 792, 17 August 1989. Data collection, record keeping, training and facility records at Wildlife International, Ltd. complied with Good Laboratory Practices, with the following exceptions:

The test substance was not characterized in accordance with full GLP compliance prior to the study; however, the characterization was performed according to 3M Standard Operating Procedures and Methods, and all raw data are being maintained in the 3M archives. The test substance has been recharacterized in accordance with GLP on September 7, 2000.

The stability of the test substance under conditions of storage at the test site was not determined in accordance with Good Laboratory Practice Standards.

The phase of the study conducted by University of Maryland was not conducted in compliance with Good Laboratory Practices.

STUDY DIRECTOR:

Susan J. Palmer
Senior Biologist

4-6-01
DATE

SPONSOR APPROVAL:

Sponsor

5/15/01
DATE

QUALITY ASSURANCE STATEMENT

This study was examined for compliance with Good Laboratory Practice Standards as published by the U.S. Environmental Protection Agency, 40 CFR Parts 160 and 792, 17 August 1989. The dates of all inspections and audits and the dates that any findings were reported to the Study Director and Laboratory Management were as follows:

		DATE REPORTED TO:	
ACTIVITY:	DATE CONDUCTED:	STUDY DIRECTOR:	MANAGEMENT:
Test Substance Preparation	May 12, 2000	May 12, 2000	May 18, 2000
Analytical Data and Draft Report	July 12 & 13, 2000	July 13, 2000	July 14, 2000

Timothy A. Springer, Ph.D.
Manager, Regulatory and Technical Support

4/6/01
DATE

REPORT APPROVAL

SPONSOR: 3M Corporation

TITLE: PFOS: A Frog Embryo Teratogenesis Assay – *Xenopus* (FETAX)

WILDLIFE INTERNATIONAL, LTD. PROJECT NUMBER: 454A-116

STUDY DIRECTOR:

Susan J Palmer — 4-6-01

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Director, Aquatic Toxicology and
Non-Target Plants

DATE

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SUMMARY

SPONSOR:	3M Corporation
SPONSOR'S REPRESENTATIVE:	Rochelle R. Robideau
LOCATION OF STUDY, RAW DATA AND A COPY OF THE FINAL REPORT:	Wildlife International, Ltd. Easton, Maryland 21601

WILDLIFE INTERNATIONAL, LTD. PROJECT NUMBER:	454A-116
STUDY:	PFOS: A Frog Embryo Teratogenesis Assay – *Xenopus* (FETAX)
TEST SUBSTANCE:	PFOS (Perfluorooctanesulfonate, Potassium Salt) IUPAC Name: 1-Octanesulfonic acid,1,1,2,2,3,3,4,4,5,5,6,6,7,7,8,8,8-heptadecafluoro-potassium salt; CAS #2795-39-3
REFERENCE SUBSTANCE:	6-aminonicotinamide
NOMINAL TEST CONCENTRATIONS:	Negative Control, 1.82, 3.07, 5.19, 8.64, 14.4 and 24.0 mg a.i./L and 5.5 and 2500 mg/L 6-aminonicotinamide
MEAN MEASURED TEST CONCENTRATIONS:	1st Assay: Negative Control, 2.00, 2.83, 4.73, 7.90, 14.7 and 24.6 mg a.i./L 2nd Assay: Negative Control, 1.91, 3.04, 4.82, 7.97, 13.3 and 23.1 mg a.i./L 3rd Assay: Negative Control, 1.93, 3.27, 5.25, 8.26, 14.0 and 23.9 mg a.i./L
TEST DATES:	1st Assay: Experimental Start – May 15, 2000 Biological Termination – May 19, 2000 Experimental Termination – May 22, 2000 2nd and 3rd Assays: Experimental Start – May 22, 2000 Biological Termination – May 26, 2000 Experimental Termination – May 30, 2000
LENGTH OF EXPOSURE:	96 Hours

TEST ORGANISM:	South African Clawed Frog (*Xenopus laevis*)
SOURCE OF TEST ORGANISMS:	University of Maryland Wye Research and Education Center Queenstown, Maryland 21658
AGE OF TEST ORGANISMS:	Embryos

SUMMARY

- Continued -

RESULTS (BASED ON MEAN MEASURED CONCENTRATIONS):	1st Assay:	2nd Assay:	3rd Assay:
96-HOUR LC_{50}:	13.8 mg a.i./L	17.6 mg a.i./L	15.3 mg a.i./L
95% CONFIDENCE LIMITS:	12.38 – 15.31 mg a.i./L	15.53 – 19.96 mg a.i./L	13.13 – 17.75 mg a.i./L
96-HOUR EC_{50}:	12.1 mg a.i./L	17.6 mg a.i./L	16.8 mg a.i./L
95% CONFIDENCE LIMITS:	10.00 – 14.58 mg a.i./L	13.49 – 22.88 mg a.i./L	12.35 – 22.82 mg a.i./L
MINIMUM CONCENTRATION TO INHIBIT GROWTH (MCIG):	Not calculable	7.97 mg a.i./L	8.26 mg a.i./L
TERATOGENIC INDEX (TI):	1.1	1.0	0.9

INTRODUCTION

This study was conducted in three assays by Wildlife International, Ltd. for 3M Corporation at the University of Maryland Wye Research and Education Center (UM-WREC) in Queenstown, Maryland and at the Wildlife International, Ltd. aquatic toxicology facility in Easton, Maryland. The in-life phase of the first assay was conducted from May 15 to May 19, 2000, while the second and third assays were concurrently conducted from May 22 to May 26, 2000. Raw data generated by Wildlife International, Ltd., data obtained from the University of Maryland, and a copy of the final report are filed under Project Number 454A-116 in archives located on the Wildlife International, Ltd. site.

OBJECTIVE

The objective of this study was to determine the possible developmental toxicity of PFOS (Perfluorooctanesulfonate, Potassium Salt), also referred to as test substance U2723, on the embryos of the South African clawed frog (*Xenopus laevis*) during a 96-hour exposure period.

EXPERIMENTAL DESIGN

Three definitive assays were conducted with PFOS using the frog embryo teratogenesis assay – *Xenopus* (FETAX). During each assay, *Xenopus* embryos were exposed to a geometric series of six test concentrations (PFOS in FETAX solution), a negative control (FETAX solution only), and two concentrations of a reference toxicant (6-aminonicotinamide in FETAX solution) for 96 hours. Two replicate test chambers were maintained in each treatment group and four replicate test chambers were maintained in each control group. Each test chamber contained 25 embryos for a total of 50 embryos per treatment group and 100 embryos per control group. An abiotic replicate at the highest concentration was included in each assay for analytical sampling at 96 hours.

Nominal test concentrations were selected in consultation with the Sponsor, and were based upon the results of an exploratory range finding toxicity test. Nominal test concentrations selected were 1.82, 3.07, 5.19, 8.64, 14.4 and 24.0 mg active ingredient (a.i.)/L. Mean measured test concentrations were determined at Wildlife International, Ltd. from samples of test solution collected from each treatment and the negative control group prior to and at the end of each assay.

The test solutions were prepared at Wildlife International, Ltd. and were delivered to UM-WREC prior to each assay. At UM-WREC, the embryos were randomly assigned to test chambers containing the appropriate FETAX solution and were observed for 96 hours. The FETAX solutions in the test chambers were renewed every 24 hours, and mortality observations and general water quality measurements were made at each 24-hour renewal period. At the end of the 96-hour exposure period, each surviving embryo was examined for malformations using a dissecting microscope. An LC50 value for mortality and an EC50 value for malformation were calculated at 96 hours for each assay. When possible, the minimum concentration to inhibit growth (MCIG) and the teratogenic index (TI) were calculated for each assay.

MATERIALS AND METHODS

The study was conducted based on the procedures outlined in the protocol, "PFOS: A Frog Embryo Teratogenesis Assay – Xenopus (FETAX)". The protocol was based on procedures outlined in ASTM Standard E1439-91 *Standard Guide for Conducting the Frog Embryo Teratogenesis Assay – Xenopus (FETAX)* (1) and ASTM Standard E729-88a *Standard Guide for Conducting Acute Toxicity Tests with Fishes, Macroinvertebrates and Amphibians* (2).

Test and Reference Substances

The test substance was received from 3M Corporation on October 29, 1998 and was assigned Wildlife International, Ltd. identification number 4675. The test substance was described as a white powder. It was identified as FC-95 from lot number 217 (T-6295). Information provided by the Sponsor indicated a purity of 98.9%, and an expiration date of 2008. The test substance was reanalyzed by the Sponsor and the Certificate of Analysis dated September 7, 2000 indicated a purity of 86.9% and an expiration date of August 31, 2001. The test substance was stored at ambient room temperature.

The reference substance was maintained by UM-WREC. It was identified as 6-aminonicotinamide (CAS #329-89-5; Sigma; Lot No. 125H0006).

Preparation of Test Concentrations

Prior to each assay, FETAX solution was prepared at UM-WREC and delivered to Wildlife International, Ltd. for the preparation of the stock and test solutions. The second and third assays were conducted concurrently using common test solutions. A primary stock solution was prepared in FETAX solution at a concentration of 48 mg a.i./L. The primary stock solution was mixed by sonication and stirring to aid in the solubilization of the

test substance. The primary stock was proportionally diluted with FETAX solution to prepare the six test concentrations. Nominal test concentrations were 1.82, 3.07, 5.19, 8.64, 14.4 and 24.0 mg a.i./L, based on a test substance purity of 86.9%. The test solutions were delivered to UM-WREC prior to the start of each assay. The reference toxicant solutions were prepared at UM-WREC.

Analytical Chemistry

Samples were collected from each test solution and the negative control solution at preparation, and at the termination of each assay (96 hours) to measure concentrations of the test substance. Samples of test solution from the abiotic replicates also were collected at 96 hours. The samples were analyzed at Wildlife International, Ltd. Analytical procedures used in the analysis of the samples are provided in Appendix II.

In-Life Phase of Assays

Details of the in-life phase of the three assays conducted at UM-WREC are included in Appendix I.

RESULTS AND DISCUSSION

Measurement of Test Concentrations

Results of analyses to measure concentrations of PFOS in FETAX solution samples collected during the three assays are presented in Table 1 and in the analytical chemistry report (Appendix II). Nominal concentrations selected for use in this study were 1.82, 3.07, 5.19, 8.64, 14.4 and 24.0 mg a.i./L. In the first assay, the measured concentrations of PFOS in test solution samples collected prior to initiation of exposure of the test organisms ranged from 112 to 141% of the nominal concentrations. Samples collected at test termination had a measured concentration range of 54.7 to 98.6% of nominal values. The sample from the abiotic 24.0 mg a.i./L treatment group was comparable to samples from the 24.0 mg a.i./L treatment group with the frog embryos present.

For the second and third assays, common test solutions were prepared. The measured concentrations of PFOS in test solution samples collected prior to initiation of exposure of the test organisms ranged from 95.8 to 117% of the nominal concentrations. In the second assay, samples collected at test termination had a measured concentration range of 80.7 to 112% of nominal values. The sample from the abiotic 24.0 mg a.i./L treatment group was comparable to samples from the 24.0 mg a.i./L treatment group with the frog embryos present. In the third assay, samples collected at test termination had a measured concentration range of 93.4 to 114% of nominal values. The sample from the abiotic 24.0 mg a.i./L treatment group was comparable to samples from the 24.0 mg a.i./L treatment group with the frog embryos present.

When measured concentrations of the samples analyzed prior to and at the end of each assay were averaged, the mean measured concentrations for the first assay were 2.00, 2.83, 4.73, 7.90, 14.7 and 24.6 mg a.i./L, representing 110, 92.2, 91.1, 91.4, 102 and 103% of nominal concentrations, respectively. The mean measured concentrations for the second assay were 1.91, 3.04, 4.82, 7.97, 13.3 and 23.1 mg a.i./L, representing 105, 99.0, 92.9, 92.2, 92.4 and 96.3% of nominal concentrations, respectively. The mean measured concentrations for the third assay were 1.93, 3.27, 5.25, 8.26, 14.0 and 23.9 mg a.i./L, representing 106, 107, 101, 95.6, 97.2 and 99.6% of nominal concentrations, respectively. The results of the study were based on the mean measured test concentrations for each assay.

Definitive Assay Results

The results of the three definitive FETAX assays conducted with PFOS at the UM-WREC are included in Appendix I and are summarized in Table 2. Exposure to PFOS caused significant embryo mortality at the two highest test concentrations (14.4 and 24.0 mg a.i./L nominal concentration) in each of the three assays. Most of the embryo mortality appeared to be caused by the gut coiling through the body wall at the two highest concentrations. Embryo survival was ≥90% at all test concentrations up to 8.64 mg a.i./L nominal concentration. The 96-hour LC50 values for PFOS ranged from 13.8 to 17.6 mg a.i./L mean measured concentration in the three assays (Table 2).

There was a positive correlation between PFOS exposure and malformations in each of the three assays, with increasing concentrations causing an increase in the incidence of malformed embryos. The 96-hour EC50 values, based on the incidence of malformed embryos, ranged from 12.1 to 17.6 mg a.i./L mean measured concentration in the three assays (Table 2). The most common types of malformations caused by exposure to PFOS were improper gut coiling, edema, notochord abnormalities and facial abnormalities.

Growth of the embryos was not effected by exposure to PFOS in the first assay. In the second and third assays, the minimum concentration inhibiting growth (MCIG) was 8.64 mg a.i./L nominal concentration. This reduction in growth may be attributed more to the increased incidence of malformed embryos in the test concentrations than to an overall decrease in growth. The teratogenic index (TI), defined as the 96-hour LC50 value for mortality divided by the 96-hour EC50 value for malformed embryos, provides an estimate of the teratogenic risk associated with PFOS. The TI's in the three assays ranged from 0.9 to 1.1, indicating the PFOS has a low potential to be a developmental hazard.

CONCLUSIONS

The 96-hour LC50 value for mortality for *Xenopus laevis* exposed to PFOS in each of three assays was 13.8, 17.6 and 15.3 mg a.i./L mean measured concentration, respectively. The 96-hour EC50 value for embryo malformation in each of three assays was 12.1, 17.6 and 16.8 mg a.i./L mean measured concentration, respectively. The MCIG, determined in the second and third assays, was 7.97 and 8.26 mg a.i./L mean measured concentration, respectively. The TI's ranged from 0.9 to 1.1 in the three assays, indicating the PFOS has a low potential to be a developmental hazard.

REFERENCES

1 **ASTM Standard E1439-91**. 1998. *Standard Guide for Conducting the Frog Embryo Teratogenesis Assay – Xenopus (FETAX)*. American Society for Testing and Materials. Philadelphia, Pennsylvania.

2 **ASTM Standard E729-88a**. 1994. *Standard Guide for Conducting Acute Toxicity Tests with Fishes, Macroinvertebrates, and Amphibians*. American Society for Testing and Materials. Philadelphia, Pennsylvania.

Table 1

Summary of Analytical Chemistry Data

Nominal Test Concentration (mg a.i./L)	Sampling Time (Day)	1st Assay			2nd Assay[1]			3rd Assay[1]		
		Measured Concentration (mg a.i./L)	Mean Measured Concentration (mg a.i./L)	Percent of Nominal	Measured Concentration (mg a.i./L)	Mean Measured Concentration (mg a.i./L)	Percent of Nominal	Measured Concentration (mg a.i./L)	Mean Measured Concentration (mg a.i./L)	Percent of Nominal
Negative Control	0[2]	<LOQ[4]	<LOQ	--	<LOQ	<LOQ	--	<LOQ	<LOQ	--
	4[3]	<LOQ			<LOQ			<LOQ		
1.82	0	2.58	2.00	110	1.77	1.91	105	1.77	1.93	106
	4	1.42			2.04			2.08		
3.07	0	3.94	2.83	92.2	3.59	3.04	99.0	3.59	3.27	107
	4	1.72			2.49			2.94		
5.19	0	6.62	4.73	91.1	5.45	4.82	92.9	5.45	5.25	101
	4	2.84			4.18			5.05		
8.64	0	10.7	7.90	91.4	8.43	7.97	92.2	8.43	8.26	95.6
	4	5.09			7.51			8.09		
14.4	0	18.5	14.7	102	14.5	13.3	92.4	14.5	14.0	97.2
	4	10.8			12.1			13.5		
24.0	0	26.9	24.6	103	23.0	23.1	96.3	23.0	23.9	99.6
	4	22.3			23.1			24.7		
24.0 (abiotic)	4	23.7	--	98.6	23.9	--	99.6	24.1	--	100

[1] The 2nd and 3rd assays were conducted concurrently using common batches of test solution prepared for Day 0.
[2] Day 0 samples were collected from the test solutions at preparation prior to transfer to UM-WREC.
[3] Day 4 samples were collected from the test solutions at the termination of each assay at UM-WREC and transferred to Wildlife International, Ltd. for analysis.
[4] The limit of quantitation (LOQ) was 0.240 mg a.i./L.

Table 2

LC50, EC50, MCIG and TI Values [1]

Parameter	1st Assay	2nd Assay	3rd Assay
96-Hour LC50 (mg a.i./L):	13.8	17.6	15.3
95% Confidence Limits (mg a.i./L):	12.38 – 15.31	15.53 – 19.96	13.13 – 17.75
96-Hour EC50 (mg a.i./L):	12.1	17.6	16.8
95% Confidence Limits (mg a.i./L):	10.00 – 14.58	13.49 – 22.88	12.35 – 22.82
Minimum Concentration Inhibiting Growth (MCIG) (mg a.i./L):	--[2]	7.97	8.26
Teratogenic Index (TI):	1.1	1.0	0.9

[1] Values are based on the mean measured concentrations for each assay.
[2] The MCIG could not be calculated because PFOS had no effect on embryonic growth at any of the test concentrations with surviving embryos in the 1st assay.

APPENDIX I

Certificate of Analysis

INTERIM CERTIFICATE OF ANALYSIS

Revision 1(9/7/00)

Centre Analytical Laboratories COA Reference #: 023-018A

3M Product: PFOS, Lot 217

Reference #: SD-018

Purity: 86.9%

Test Name	Specifications	Result
Purity[1]		86.9%
Appearance	White Crystalline Powder	Conforms
Identification NMR		Positive
Metals (ICP/MS) 1. Calcium 2. Magnesium 3. Sodium 4. Potassium[2] 5. Nickel 6. Iron 7. Manganese		 1. 0.005 wt./wt.% 2. 0.001 wt./wt.% 3. 1.439 wt./wt.% 4. 6.849 wt./wt.% 5. <0.001 wt./wt.% 6. 0.005 wt./wt.% 7. <0.001 wt./wt.%
Total % Impurity (NMR)		1.93 wt./wt.%
Total % Impurity (LC/MS)		8.41 wt./wt.%
Total % Impurity (GC/MS)		None Detected
Related Compounds – POAA		0.33 wt./wt.%
Residual Solvents (TGA)		None Detected
Purity by DSC		Not Applicable[3]
Inorganic Anions (IC) 1. Chloride 2. Fluoride 3. Bromide 4. Nitrate 5. Nitrite 6. Phosphate 7. Sulfate[4]		 1. <0.015 wt./wt.% 2. 0.59 wt./wt.% 3. <0.040 wt./wt.% 4. <0.009 wt./wt.% 5. <0.006 wt./wt.% 6. <0.007 wt./wt.% 7. 8.76 wt./wt.%
Organic Acids [5] (IC) 1. TFA 2. PFPA 3. HFBA 4. NFPA		 1. <0.1 wt./wt.% 2. <0.1 wt./wt.% 3. 0.10 wt./wt.% 4. 0.28 wt./wt.%
Elemental Analysis[6]: 1. Carbon 2. Hydrogen 3. Nitrogen 4. Sulfur 5. Fluorine	 1. Theoretical Value = 17.8% 2. Theoretical Value = 0% 3. Theoretical Value = 0% 4. Theoretical Value = 5.95% 5. Theoretical Value = 60%	 1. 12.48 wt./wt.% 2. 0.244 wt./wt.% 3. 1.74 wt./wt.% 4. 8.84 wt./wt.% 5. 54.1 wt./wt.%

INTERIM CERTIFICATE OF ANALYSIS

Centre Analytical Laboratories COA Reference #: 023-018A

Date of Last Analysis: 08/31/00

Expiration Date: 08/31/01

Storage Conditions: Frozen ≤-10°C

Re-assessment Date: 08/31/01

[1]Purity = 100% - (sum of metal impurities, 1.45% +LC/MS impurities, 8.41%+Inorganic Fluoride, 0.59%+NMR impurities, 1.93%+organic acid impurities, 0.38%+POAA, 0.33%)

Total impurity from all tests = 13.09%
Purity = 100% - 13.09% = 86.9%

[2]Potassium is expected in this salt form and is therefore not considered an impurity.

[3]Purity by DSC is generally not applicable to materials of low purity. No endotherm was observed for this sample.

[4]Sulfur in the sample appears to be converted to SO_4 and hence detected using the inorganic anion method conditions. The anion result agrees well with the sulfur determination in the elemental analysis, lending confidence to this interpretation. Based on the results, the SO_4 is not considered an impurity.

[5]TFA	Trifluoroacetic acid
HFBA	Heptafluorobutyric acid
NFPA	Nonofluoropentanoic acid
PFPA	Pentafluoropropanoic acid

[6]Theoretical value calculations based on the empirical formula, $C_8F_{17}SO_3^-K^+$ (MW=538)

This work was conducted under EPA Good Laboratory Practice Standards (40 CFR 160).

INTERIM CERTIFICATE OF ANALYSIS

Centre Analytical Laboratories COA Reference #: 023-018A

LC/MS Purity Profile:

Impurity	wt./wt. %
C4	1.22
C5	1.33
C6	4.72
C7	1.14
Total	**8.41**

Note: The C4 and C6 values were calculated using the C4 and C6 standard calibration curves, respectively. The C5 value was calculated using the average response factors from the C4 and C6 standard curves. Likewise, the C7 value was calculated using the average response factors from the C6 and C8 standard curves.

Prepared By: _____ _____

David S. Bell — Date

Scientist, Centre Analytical Laboratories

Reviewed By: _____ _____

John Flaherty — Date

Laboratory Manager, Centre Analytical Laboratories

APPENDIX II

Evaluation of the Toxicity and Teratogenicity of Chemical U2723
Using the Frog Embryo Teratogenesis Assay – *Xenopus* (FETAX)

Report No.
WREC-00-02

EVALUATION OF THE TOXICITY AND TERATOGENICITY OF CHEMICAL U2723 USING THE FROG EMBRYO TERATOGENESIS ASSAY-*XENOPUS* (FETAX)

Final Report

Prepared for

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Prepared by

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July 15, 2000

Revision Date:

March 19, 2001

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1. INTRODUCTION

The evaluation of the potential toxicity and teratogenicity of chemical U2723 was initiated at the request of Mr. David A. Palmer, Wildlife International, Ltd., Easton, Maryland. The study was conducted by Mr. Steven D. Turley and Dr. Dennis T. Burton of the University of Maryland Wye Research and Education Center, Queenstown, Maryland. The test program was performed in accordance with the "Revised Statement of Work for Evaluating the Potential Teratogenicity of U2723 Using 96-Hour Frog Embryo Teratogenesis Assay-*Xenopus* (FETAX)", submitted to and approved by Wildlife International, Ltd., March 30, 2000 (Turley, 2000). The potential toxicity and teratogenicity of chemical U2723 was evaluated by the Frog Embryo Teratogenesis Assay-*Xenopus* (FETAX). Three 96-h definitive FETAX assays were conducted on chemical U2723.

2. MATERIALS AND METHODS

Three definitive FETAX assays were conducted on chemical U2723 using the frog embryo teratogenesis assay-*Xenopus* (FETAX). The assay is a 96-h quantitative developmental assay used to screen for developmental toxicants in aquatic media. The assays were conducted using the static renewal (solutions renewed every 24 h) test protocol Designation E 1439-91 of the American Society for Testing and Materials (ASTM, 1998). Embryolethality (96-h LC50), malformations (96-h EC50), and growth retardation were used as endpoints in the assays.

Embryos were obtained from breeding colonies of *Xenopus laevis* at the University of Maryland Wye Research and Education Center (UMD/WREC). The UMD/WREC adult colonies were maintained in flow-through (~4 replacement volumes per day) circular polyethylene aquaria (0.91 m I.D. x 0.36 m high) with a water depth of 10 cm. Each aquarium contained a maximum of 10 adults. UMD/WREC non-chlorinated deep well water held at 23.5 ± 0.5 °C served as the culture medium. All frogs were fed every 5-6 d with commercial beef liver supplemented with liquid vitamins (PolyViSol™; Mead-Johnson Nutritionals, Evansville, Indiana). The colony was held under a photoperiod of 16 h light:8 h dark. Mating pairs were bred in the dark in 23.5 ± 0.5 °C UMD/WREC non-chlorinated water at ~70 d intervals by injecting 400 and 800 I.U. of human chorionic gonadotropin (HCG) in the dorsal lymph sac of the males and females, respectively. Amplexus occurred 4-6 h after injecting HCG; egg deposition occurred 9-12 h following HCG injection.

Embryos between normal stage 8 blastulae and normal stage 11 gastrulae were used to initiate the assays (Nieuwkoop and Faber, 1975). Embryos from separate breeding pairs were used for each test to check for variability in the sensitivity of individual clutches of embryos. All embryos were examined two times for viability. The embryos were de-jellied in a 2% L-cysteine solution (2 g of L-cysteine per 98 mL of FETAX solution). Once de-jellied, the embryos were rinsed and re-suspended in FETAX solution (ASTM, 1998). The embryos were assayed in covered 60 mm glass Petri dishes containing 10 mL of solution. All embryos were randomly assigned to the Petri dishes. Two replicates of 25 embryos/replicate were used for each of six assay treatments; four replicates were used for the controls as required by the test protocol (ASTM, 1991). The assays were conducted at 24 ± 0.2°C under a 12-h light: 12-h dark photoperiod (fluorescent lights; ~75 foot candles at the surface of the test medium) in a constant temperature environmental chamber. All Petri dishes were randomly placed in the environmental chamber used for the assays. Wildlife International, Ltd., personnel prepared and delivered stock solutions of each concentration at the start of each assay. The concentration of each solution was quantified at Wildlife International, Ltd. Two concentrations of the reference toxicant, 6-aminonicotinamide (CAS #329-89-5; Sigma; Lot No. 125H0006), were used at each assay period as outlined in the ASTM (1998) protocol.

Mortality observations and general water quality measurements were made at each 24-h renewal period. All dead embryos were removed at each observation period. At the end of the 96-h exposure period, each surviving embryo was examined for malformations using a dissecting microscope. The identification and interpretation of malformations in the embryos at 96 h were made via the atlas of Bantle et al. (1991). The head-tail length of each surviving embryo was determined via Sigma Scan's digitizing software (SPSS, Corte Madera, California). A random group of normal embryos and embryos with each type of malformation were preserved in 10% buffered formalin (pH 7) for photographing.

The Trimmed Spearman-Karber statistical procedure was used to determine the 96-h LC50 for mortality and 96-h EC50 for malformations. The minimum concentration to inhibit growth (MCIG) was determined by Bonferroni's T-Test. All statistical tests were performed using Toxstat (WEST and Gulley, 1994). A minimum probability level of 0.05 was used. The teratogenic index (TI) was calculated by dividing the LC50 by the EC50. The test acceptability criteria given in the ASTM (1998) test protocol was used for each definitive test.

3. RESULTS AND DISCUSSION

The data for definitive assays 1, 2, and 3 are summarized in Appendices 1,2, and 3, respectively. Copies of the raw data sheets and 6-aminonicotinamide reference toxicant data for assays 1, 2, and 3 are given in Appendices 4, 5, and 6, respectively. The nominal concentrations of 1.9, 3.2, 5.4, 9.0, 15.0 and 25.0 mg/L on the raw data sheets correspond to the nominal concentrations of 1.82, 3.07, 5.19, 8.64, 14.4 and 24.0 mg a.i./L in the report. Photographs of normal and malformed embryos of each type observed in the study are given in Attachment 1. Table 1 is summary of the endpoint parameters established in the three definitive assays.

Table 1. Summary of Data for the Three Assays with Chemical U2723[a]

Parameter	Assay 1	Assay 2	Assay 3
LC50 (95% C. I.)	13.8 (12.38 - 15.31)	17.6 (15.53 - 19.96)	15.3 (13.13 - 17.75)
EC50 (95% C. I.)	12.1 (10.00 - 14.58)	17.6 (13.49 - 22.88)	16.8 (12.35 - 22.82)
MCIG	[b]	7.97	8.26
TI	1.1	1.0	0.9

[a] The LC50, EC50, and MCIG values are based on mean measured concentrations of chemical U2723 (mg a.i./L) for each assay.
[b] The MCIG could not be calculated because chemical U2723 had no effect on embryonic growth at any of the test treatments with surviving embryos (Appendix A; Table A1- 4).

Exposure to chemical U2723 caused significant embryo mortality at the two highest treatment concentrations (14.4 and 24.0 mg/L) in each of the three assays. Most of the embryo mortality appeared to be caused by the gut coiling through the body wall at the two highest assay treatments. Embryo survival was ≥90% at all exposure treatments up to 8.64 mg/L. The 96-h LC50s for chemical U2723 ranged from 13.8 to 17.6 mg/L (Table 1).

There was a positive correlation between chemical U2723 exposure and malformations in each of the three assays, with increasing concentrations causing an increase in the incidence of malformed embryos. The 96-h EC50s, based on the incidence of malformed embryos, ranged from 12.1 to 17.6 mg/L (Table 1). The most common types of malformations caused by exposure to chemical U2723 were: improper gut coiling,

edema, notochord abnormalities and facial abnormalities.

Growth of the embryos was not affected by exposure to chemical U2723 in assay 1. In assays 2 and 3 , the minimum concentration inhibiting growth (MCIG) was 7.97 and 8.26 mg/L, respectively. This reduction in growth may be attributed more to the increased incidence of malformed embryos in these test concentrations than an overall decrease in growth. Malformed embryos are generally smaller than normally developing embryos.

The teratogenic index (TI), which by definition is the 96-h LC50 divided by the 96-h EC50 (malformed embryos), provides an estimate of the teratogenic risk associated with a material (Dumont et al., 1983). TI values of 1.5 to 2.0 indicate that a material may be a potential teratogen. Materials with TI values >2.0 should be considered for further teratogenicity testing. The TI's in the current study ranged from 0.9 to 1.1 which indicate that chemical U2723 has a low potential to be a developmental hazard.

The three assays met all of the ASTM (1998) protocol test acceptability criteria, with one exception. The reference toxicant (6-aminonicotinamide) high concentration of 2,500 mg/L did not meet the ASTM protocol test acceptability criterion. A concentration of 2,500 mg/L should kill between 40 and 60% of the embryos in 96 h. All embryos died at 2,500 mg/L in all three reference toxicant tests. All three reference toxicant tests met the protocol test acceptability criteria for the low reference toxicant concentration of 5.5 mg/L which should cause between 40 and 60% malformed embryos after 96 h of exposure. The 5.5 mg/L reference toxicant tests produced an average of 55.6, 55.3, and 53.1% malformed embryos during assays 1, 2, and 3, respectively. The results of the reference toxicant concentrations in the three current tests are consistent and not at variance with previous experience in our laboratory. The survival of control embryos after 96 hours in our laboratory consistently meet the ASTM protocol acceptability criterion of >90% survival. In the current chemical U2723 assays, the average survival of the controls was 99, 99, and 100%, in assays 1, 2, and 3, respectively.

The chain of custody forms for delivery of the assay solutions from Wildlife International, Ltd. to the UMD/WREC were retained by Wildlife International, Ltd. Thus, copies of the chain of custody forms are not included in this report.

4. REFERENCES

ASTM. 1998. Standard guide for conducting the frog embryo teratogenesis assay-*Xenopus* (FETAX). ASTM Designation E 1439-91. 1998 Annual Book of ASTM Standards Section 11 Water and Environmental Technology, Amer. Soc. Testing Materials, Philadelphia, PA.

Bantle, J.A., J.N. Dumont, R.A. Finch, and G. Linder. 1991. Atlas of abnormalities. A guide for the performance of FETAX. Oklahoma State Univ., Stillwater, OK.

Dumont, J., T. Schultz, M. Buchanan, and G. Kao. 1983. Frog embryo teratogenesis assay-*Xenopus* (FETAX) - A short-term assay applicable to complex environmental mixtures. Pages 393-405 *In*: Waters, Sandhu, S.S., J. Lewtas, L. Claxton, N. Chernoff, and S. Nesnow, eds. Short-term bioassays in the analysis of complex environmental mixtures III, Plenum, New York, NY.

Nieuwkoop, P.D. and J. Faber. 1975. Normal tables of *Xenopus laevis* (Daudin), 2nd ed. North Holland, Amsterdam, Netherlands.

Turley, S.D. 2000. Revised statement of work for evaluating the potential teratogenicity of U2723 using 96-hour frog embryo teratogenesis assay-*Xenopus* (FETAX). March 30, 2000. University of Maryland Wye Research and Education Center, Queenstown, MD.

WEST (Western Ecosystem Technology, Inc.) and Gulley, D. D. 1994. Toxstat, v. 3.4. Western Ecosystem Technology, Inc., Cheyenne, WY.

APPENDIX 1

FROG EMBRYO TERATOGENESIS ASSAY-*XENOPUS* (FETAX) CONDUCTED WITH CHEMICAL U2723 (ASSAY 1)

Test Method:	ASTM Designation E 1439-91 ASTM (1998)
Type of Test:	Static renewal (every 24 h)
Date:	May 15-19, 2000
Investigator:	S. D. Turley
Laboratory:	UMD/WREC
Chemical:	
Designation:	U2723
Source:	Wildlife International, Ltd.
General Water Quality:	See Table A1-1
Test Medium:	FETAX solution
Test Organism:	
Scientific Name:	*Xenopus laevis*
Age at Start of Test:	Stage 8 blastula to stage 11 gastrula
Source:	UMD/WREC culture
Experimental Chambers:	
Material:	Glass petri dishes
Test Solution Volume:	10 mL
No. Organisms/Replicate:	25
No. Organisms/Treatment:	Control: 100 Treatment: 50
Lighting:	Fluorescent; 60-85 foot candles
Endpoints:	Mortality; malformation; growth
Test Temperature:	24 ± 2°C

Results:

Note: For Assay 1, statistical calculations are based on the mean measured concentrations of 2.00, 2.83, 4.73, 7.90, 14.70 and 24.60 mg a.i./L, rather than on the nominal concentrations.

Mortality:

The 96-h LC50 = 13.8 mg a.i./L (95% confidence limits = 12.38 - 15.31).

The mortality data are summarized in Table A1-2.

Malformations:

The 96-h EC50 (malformed embryos) = 12.1 mg a.i./L (95% confidence limits = 10.00 - 14.58).

The number of malformed embryos are summarized in Table A1-2. The types of malformations are summarized in Table A1-3.

Growth:

Chemical U2723 had no effect on embryonic growth at any of the test treatments with surviving embryos. As a result, the minimum concentration inhibiting growth (MCIG) could not be calculated.

The growth data are summarized in Table A1-4.

Teratogenic Index:

The teratogenic index (TI) = 1.1.

Table A1-1. Summary of The General Water Chemistry Data for Chemical U2723 - Assay 1 - Dissolved Oxygen (mg/L)

	Test Concentrations (mg/L)						
	0.00	1.82	3.07	5.19	8.64	14.40	24.00
Day 0							
0 H	8.3	8.4	8.4	8.4	8.4	8.4	8.5
Day 1							
24 H	8.0	7.8	7.9	7.9	8.0	8.0	7.9
0 H	8.4	8.4	8.5	8.4	8.5	8.5	8.4
Day 2							
24 H	7.7	7.7	7.6	7.7	7.8	7.8	7.8
0 H	8.3	8.3	8.4	8.3	8.2	8.3	8.3
Day 3							
24 H	7.4	7.5	7.7	7.7	7.8	7.9	7.9
0 H	8.3	8.2	8.1	8.2	8.1	8.1	8.1
Day 4							
24 H	7.7	7.7	7.6	7.5	7.7	7.7	7.7
Mean	8.1	8.0	8.1	8.1	8.1	8.1	8.1
Min	7.4	7.5	7.6	7.7	7.8	7.8	7.8
Max	8.4	8.4	8.5	8.4	8.5	8.5	8.5

Table A1-1. (Continued) - pH (Standard Units)

	Test Concentrations (mg/L)						
	0.00	1.82	3.07	5.19	8.64	14.40	24.00
Day 0							
0 H	7.74	7.78	7.79	7.78	7.78	7.76	7.62
Day 1							
24 H	7.26	7.24	7.30	7.34	7.35	7.34	7.33
0 H	7.24	7.45	7.38	7.43	7.43	7.44	7.32
Day 2							
24 H	7.16	7.17	7.20	7.23	7.23	7.23	7.25
0 H	7.22	7.25	7.28	7.32	7.34	7.35	7.27
Day 3							
24 H	7.11	7.13	7.16	7.17	7.25	7.18	6.95
0 H	7.13	7.20	7.23	7.25	7.26	7.26	7.18
Day 4							
24 H	7.11	7.15	7.18	7.19	7.18	7.20	7.19
Min	7.11	7.13	7.16	7.17	7.18	7.18	6.95
Max	7.74	7.78	7.79	7.78	7.78	7.76	7.62

Table A1-1. (Continued) - Temperature (°C)

	Test Concentrations (mg/L)						
	0.00	1.82	3.07	5.19	8.64	14.40	24.00
Day 0							
0 H	24.0	24.0	24.0	24.0	24.0	24.0	24.0
Day 1							
24 H	24.0	24.0	24.0	24.0	24.0	24.0	24.0
0 H	24.0	24.0	24.0	24.0	24.0	24.0	24.0
Day 2							
24 H	24.0	24.0	24.0	24.0	24.0	24.0	24.0
0 H	24.0	24.0	24.0	24.0	24.0	24.0	24.0
Day 3							
24 H	24.0	24.0	24.0	24.0	24.0	24.0	24.0
0 H	24.0	24.0	24.0	24.0	24.0	24.0	24.0
Day 4							
24 H	24.0	24.0	24.0	24.0	24.0	24.0	24.0
Mean	24.0	24.0	24.0	24.0	24.0	24.0	24.0
Min	24.0	24.0	24.0	24.0	24.0	24.0	24.0
Max	24.0	24.0	24.0	24.0	24.0	24.0	24.0

Table A1-2. FETAX Toxicity Test Data - Percent Embryo Mortality and Malformations after 96 Hours of Exposure to Chemical U2723 - Assay 1

Concentration (mg/L)	Rep	Number Embryos Alive	Percent Mortality	Number Embryos Malformed	Percent Malformed
Control	1	24	4.0	1	4.2
	2	25	0.0	1	4.0
	3	25	0.0	1	4.0
	4	25	0.0	1	4.0
1.82	1	24	4.0	2	8.3
	2	25	0.0	2	8.0
3.07	1	24	4.0	2	8.3
	2	24	4.0	5	20.8
5.19	1	23	8.0	7	30.4
	2	22	12.0	3	13.6
8.64	1	21	16.0	4	19.0
	2	23	8.0	7	30.4
14.40	1	16	36.0	9	56.3
	2	15	40.0	11	73.3
24.00	1	0	100		
	2	0	100		

Table A1-3. FETAX Toxicity Test Data - Type and Number of Malformed Embryos after 96 Hours Exposure to Chemical U2723 - Assay 1

Malformation	Test Concentration (mg/L)															
	0 Rep				1.82 Rep		3.07 Rep		5.19 Rep		8.64 Rep		14.40 Rep		24.00 Rep	
	1	2	3	4	1	2	1	2	1	2	1	2	1	2	1	2
Severe												1				
Stunted																
Gut	1	1	1	1	1	1	2	5	7	3	4	5	7	11		
Edema:																
Multiple								2	2		2	2	4	3		
Cardiac		1					1	3	1		2	2	4	1		
Abdominal							1	3	1		2	1	3	2		
Facial								2	2		2	2	4	2		
Cephalic																
Optic								2	2		1	1	2	6		
Tail																
Notochord												1	1			
Fin																
Face	1	1		1	2	2	1	1	4	3	2	3	4	6		
Eye												1	1			
Brain																
Hemorrhage																
Cardiac						1			2				3	1		
Blisters																
Other*																
# Malformed	1	1	1	1	2	2	2	5	7	3	4	7	9	11		

* Anal edema.

Table A1-4. FETAX Toxicity Test Data - Embryonic Growth (Length) after 96 Hours Exposure to Chemical U2723 - Assay 1

Concentration (mg/L)	Rep	Number Embryos Alive	Average Length (mm)	Mean Average Length (mm)
Control	1	24	9.05	8.59
	2	25	8.40	
	3	25	8.29	
	4	25	8.62	
1.82	1	24	8.44	8.29
	2	25	8.14	
3.07	1	24	8.88	8.80
	2	24	8.72	
5.19	1	23	8.42	8.51
	2	22	8.60	
8.64	1	21	8.83	8.71
	2	23	8.58	
14.40	1	16	8.41	8.08
	2	15	7.74	
24.00	1	0		
	2	0		

APPENDIX 2

FROG EMBRYO TERATOGENESIS ASSAY-*XENOPUS* (FETAX) CONDUCTED WITH CHEMICAL U2723 (ASSAY 2)

Test Method:	ASTM Designation E 1439-91 ASTM (1998)
Type of Test:	Static renewal (every 24 h)
Date:	May 22-26, 2000
Investigator:	S. D. Turley
Laboratory:	UMD/WREC
Chemical:	
Designation:	U2723
Source:	Wildlife International, Ltd.
General Water Quality:	See Table A2-1
Test Medium:	FETAX solution
Test Organism:	
Scientific Name:	*Xenopus laevis*
Age at Start of Test:	Stage 8 blastula to stage 11 gastrula
Source:	UMD/WREC culture
Experimental Chambers:	
Material:	Glass petri dishes
Test Solution Volume:	10 mL
No. Organisms/Replicate:	25
No. Organisms/Treatment:	Control: 100 Treatment: 50
Lighting:	Fluorescent; 60-85 foot candles
Endpoints:	Mortality; malformation; growth
Test Temperature:	24 ± 2°C

Results:

Note: For Assay 2, statistical calculations are based on the mean measured concentrations of 1.91, 3.04, 4.82, 7.97, 13.30 and 23.10 mg a.i./L, rather than on the nominal concentrations.

Mortality:

The 96-h LC50 = 17.6 mg a.i./L (95% confidence limits = 15.53 -19.96).

The mortality data are summarized in Table A2-2.

Malformations:

The 96-h EC50 (malformed embryos) = 17.6 mg a.i./L (95% confidence limits = 13.49 - 22.88)

The number of malformed embryos are summarized in Table A2-2. The types of malformations are summarized in Table A2-3.

Growth:

The minimum concentration inhibiting growth (MCIG) =7.97 mg a.i./L.

The growth data are summarized in Table A2-4.

Teratogenic Index:

The teratogenic index (TI) = 1.0.

Table A2-1. Summary of The General Water Chemistry Data for Chemical U2723 - Assay 2 - Dissolved Oxygen (mg/L)

	Test Concentrations (mg/L)						
	0.00	1.82	3.07	5.19	8.64	14.40	24.00
Day 0							
0 H	8.2	8.2	8.1	8.2	8.1	8.2	8.2
Day 1							
24 H	8.3	8.3	8.0	8.1	8.1	8.1	8.2
0 H	8.2	8.2	8.2	8.2	8.1	8.1	8.2
Day 2							
24 H	7.6	7.8	7.5	7.2	7.2	7.2	7.1
0 H	8.1	8.1	8.0	8.1	8.0	8.0	8.1
Day 3							
24 H	7.5	7.6	7.5	7.6	7.7	7.3	7.2
0 H	8.1	8.2	8.1	8.1	8.0	8.1	8.0
Day 4							
24 H	7.6	7.6	7.5	7.5	7.6	7.5	7.5
Mean	8.0	8.0	7.9	7.9	7.9	7.8	7.8
Min	7.5	7.6	7.5	7.2	7.2	7.2	7.1
Max	8.3	8.3	8.2	8.2	8.1	8.2	8.2

Table A2-1. (Continued) - pH (Standard Units)

	Test Concentrations (mg/L)						
	0.00	1.82	3.07	5.19	8.64	14.40	24.00
Day 0							
0 H	7.14	7.24	7.31	7.33	7.37	7.40	7.37
Day 1							
24 H	7.12	7.17	7.21	7.23	7.24	7.26	7.27
0 H	7.11	7.21	7.28	7.35	7.35	7.39	7.34
Day 2							
24 H	7.18	7.22	7.23	7.24	7.24	7.23	7.24
0 H	7.15	7.23	7.30	7.37	7.38	7.41	7.32
Day 3							
24 H	7.20	7.20	7.22	7.25	7.24	7.23	7.25
0 H	7.17	7.21	7.26	7.34	7.36	7.40	7.35
Day 4							
24 H	7.11	7.21	7.20	7.29	7.33	7.39	7.34
Min	7.11	7.17	7.20	7.23	7.24	7.23	7.24
Max	7.20	7.24	7.31	7.37	7.38	7.41	7.37

Table A2-1. (Continued) - Temperature (°C)

	Test Concentrations (mg/L)						
	0.00	1.82	3.07	5.19	8.64	14.40	24.00
Day 0							
0 H	24.0	24.0	24.0	24.0	24.0	24.0	24.0
Day 1							
24 H	24.0	24.0	24.0	24.0	24.0	24.0	24.0
0 H	24.0	24.0	24.0	24.0	24.0	24.0	24.0
Day 2							
24 H	24.0	24.0	24.0	24.0	24.0	24.0	24.0
0 H	24.0	24.0	24.0	24.0	24.0	24.0	24.0
Day 3							
24 H	24.0	24.0	24.0	24.0	24.0	24.0	24.0
0 H	24.0	24.0	24.0	24.0	24.0	24.0	24.0
Day 4							
24 H	24.0	24.0	24.0	24.0	24.0	24.0	24.0
Mean	24.0	24.0	24.0	24.0	24.0	24.0	24.0
Min	24.0	24.0	24.0	24.0	24.0	24.0	24.0
Max	24.0	24.0	24.0	24.0	24.0	24.0	24.0

Table A2-2. FETAX Toxicity Test Data - Percent Embryo Mortality and Malformations after 96 Hours of Exposure to Chemical U2723 - Assay 2

Concentration (mg/L)	Rep	Number Embryos Alive	Percent Mortality	Number Embryos Malformed	Percent Malformed
Control	1	25	0.0	1	4.0
	2	25	0.0	2	8.0
	3	25	0.0	0	0.0
	4	24	4.0	1	4.2
1.82	1	25	0.0	2	8.0
	2	25	0.0	2	8.0
3.07	1	23	8.0	2	8.7
	2	22	12.0	0	0.0
5.19	1	24	4.0	1	4.2
	2	22	12.0	4	18.2
8.64	1	22	12.0	4	18.2
	2	23	8.0	5	21.7
14.40	1	22	12.0	4	18.2
	2	13	48.0	9	69.2
24.00	1	9	64.0	6	66.7
	2	6	76.0	4	66.7

Table A2-3. FETAX Toxicity Test Data - Type and Number of Malformed Embryos after 96 Hours Exposure to Chemical U2723 - Assay 2

Malformation	Test Concentration (mg/L)															
	0 Rep				1.62 Rep		3.07 Rep		5.19 Rep		8.64 Rep		14.40 Rep		24.00 Rep	
	1	2	3	4	1	2	1	2	1	2	1	2	1	2	1	2
Severe																
Stunted																
Gut		1			1	1	1			4	1	3	2	6	6	4
Edema:																
Multiple					2					2		1			3	
Cardiac					2					2	2	1			4	
Abdominal					2					3		1	1		3	
Facial					2				1	3		1			3	
Cephalic																
Optic					2					1					3	
Tail																
Notochord				1		1	2						1	1		
Fin																
Face	1	2				1				2	2	3	1	9	1	3
Eye																
Brain																
Hemorrhage																
Cardiac		2														
Blisters																
Other*											1			2		
# Malformed	1	2	0	1	2	2	2	0	1	4	4	5	4	9	6	4

* Anal edema.

Table A2-4. FETAX Toxicity Test Data - Embryonic Growth (Length) after 96 Hours Exposure to Chemical U2723 - Assay 2

Concentration (mg/L)	Rep	Number Embryos Alive	Average Length (mm)	Mean Average Length (mm)
Control	1	25	8.94	8.88
	2	25	8.81	
	3	25	8.90	
	4	24	8.88	
1.82	1	25	8.39	8.45
	2	25	8.50	
3.07	1	23	8.42	8.57
	2	22	8.71	
5.19	1	24	8.69	8.72
	2	22	8.74	
8.64	1	22	7.94	7.93*
	2	23	7.92	
14.40	1	22	7.89	7.51*
	2	13	7.13	
24.00	1	9	7.50	7.39*
	2	6	7.27	

* Significantly different at alpha = 0.05 (Bonferroni T-Test)

APPENDIX 3

FROG EMBRYO TERATOGENESIS ASSAY-*XENOPUS* (FETAX) CONDUCTED WITH CHEMICAL U2723 (ASSAY 3)

Test Method:	ASTM Designation E 1439-91 ASTM (1998)
Type of Test:	Static renewal (every 24 h)
Date:	May 22-26, 2000
Investigator:	S. D. Turley
Laboratory:	UMD/WREC
Chemical:	
Designation:	U2723
Source:	Wildlife International, Inc.
General Water Quality:	See Table A3-1
Test Medium:	FETAX solution
Test Organism:	
Scientific Name:	*Xenopus laevis*
Age at Start of Test:	Stage 8 blastula to stage 11 gastrula
Source:	UMD/WREC culture
Experimental Chambers:	
Material:	Glass petri dishes
Test Solution Volume:	10 mL
No. Organisms/Replicate:	25
No. Organisms/Treatment:	Control: 100 Treatment: 50
Lighting:	Fluorescent; 60-85 foot candles
Endpoints:	Mortality; malformation; growth
Test Temperature:	24 ± 2°C

Results:

Note: For Assay 3, statistical calculations are based on the mean measured concentrations of 1.93, 3.27, 5.25, 8.26, 14.00 and 23.90 mg a.i./L, rather than on nominal concentrations.

Mortality:

The 96-h LC50 = 15.3 mg a.i./L (95% confidence limits = 13.13 - 17.75).

The mortality data are summarized in Table A3-2.

Malformations:

The 96-h EC50 (malformed embryos) = 16.8 mg a.i./L (95% confidence limits = 12.35 - 22.82).

The number of malformed embryos are summarized in Table A3-2. The types of malformations are summarized in Table A3-3.

Growth:

The minimum concentration inhibiting growth (MCIG) =8.26 mg a.i./L.

The growth data are summarized in Table A3-4.

Teratogenic Index:

The teratogenic index (TI) = 0.9.

Table A3-1. Summary of The General Water Chemistry Data for Chemical U2723 - Assay 3 - Dissolved Oxygen (mg/L)

	Test Concentrations (mg/L)						
	0.00	1.82	3.07	5.19	8.64	14.40	24.00
Day 0							
0 H	8.2	8.2	8.1	8.2	8.1	8.2	8.2
Day 1							
24 H	8.3	8.3	8.0	8.2	8.2	8.2	8.3
0 H	8.2	8.2	8.2	8.2	8.1	8.1	8.2
Day 2							
24 H	7.4	7.2	7.1	7.0	7.0	7.0	7.0
0 H	8.1	8.1	8.0	8.1	8.0	8.0	8.1
Day 3							
24 H	7.3	7.1	7.1	7.0	7.0	7.1	7.0
0 H	8.1	8.2	8.1	8.1	8.0	8.1	8.0
Day 4							
24 H	7.4	7.5	7.5	7.5	7.6	7.4	7.4
Mean	7.9	7.9	7.8	7.8	7.8	7.8	7.8
Min	7.3	7.1	7.1	7.0	7.0	7.0	7.0
Max	8.3	8.3	8.2	8.2	8.2	8.2	8.3

Table A3-1. (Continued) - pH (Standard Units)

	Test Concentrations (mg/L)						
	0.00	1.82	3.07	5.19	8.64	14.40	24.00
Day 0							
0 H	7.14	7.24	7.31	7.33	7.37	7.40	7.37
Day 1							
24 H	7.18	7.19	7.15	7.14	7.15	7.14	7.16
0 H	7.11	7.21	7.28	7.35	7.35	7.39	7.34
Day 2							
24 H	7.21	7.20	7.18	7.17	7.17	7.15	7.23
0 H	7.15	7.23	7.30	7.37	7.38	7.41	7.32
Day 3							
24 H	7.23	7.21	7.25	7.26	7.24	7.28	7.25
0 H	7.17	7.21	7.26	7.34	7.36	7.40	7.35
Day 4							
24 H	7.18	7.21	7.24	7.31	7.35	7.40	7.29
Min	7.11	7.19	7.15	7.14	7.15	7.14	7.16
Max	7.23	7.24	7.31	7.37	7.38	7.41	7.37

Table A3-1. (Continued) - Temperature (°C)

	Test Concentrations (mg/L)						
	0.00	1.82	3.07	5.19	8.64	14.40	24.00
Day 0							
0 H	24.0	24.0	24.0	24.0	24.0	24.0	24.0
Day 1							
24 H	24.0	24.0	24.0	24.0	24.0	24.0	24.0
0 H	24.0	24.0	24.0	24.0	24.0	24.0	24.0
Day 2							
24 H	24.0	24.0	24.0	24.0	24.0	24.0	24.0
0 H	24.0	24.0	24.0	24.0	24.0	24.0	24.0
Day 3							
24 H	24.0	24.0	24.0	24.0	24.0	24.0	24.0
0 H	24.0	24.0	24.0	24.0	24.0	24.0	24.0
Day 4							
24 H	24.0	24.0	24.0	24.0	24.0	24.0	24.0
Mean	24.0	24.0	24.0	24.0	24.0	24.0	24.0
Min	24.0	24.0	24.0	24.0	24.0	24.0	24.0
Max	24.0	24.0	24.0	24.0	24.0	24.0	24.0

Table A3-2. FETAX Toxicity Test Data - Percent Embryo Mortality and Malformations after 96 Hours of Exposure to Chemical U2723 - Assay 3

Concentration (mg/L)	Rep	Number Embryos Alive	Percent Mortality	Number Embryos Malformed	Percent Malformed
Control	1	25	0.0	1	4.0
	2	25	0.0	1	4.0
	3	25	0.0	0	0.0
	4	25	0.0	0	0.0
1.82	1	25	0.0	1	4.0
	2	25	0.0	2	8.0
3.07	1	25	0.0	1	4.0
	2	25	0.0	1	4.0
5.19	1	25	0.0	1	4.0
	2	25	0.0	2	8.0
8.64	1	23	8.0	1	4.3
	2	20	20.0	5	25.0
14.40	1	14	44.0	4	28.6
	2	14	44.0	7	50.0
24.00	1	6	76.0	5	83.3
	2	5	80.0	3	60.0

Table A3-3. FETAX Toxicity Test Data - Type and Number of Malformed Embryos after 96 Hours Exposure to Chemical U2723 - Assay 3

Malformation	Test Concentration (mg/L)															
	0 Rep				1.82 Rep		3.07 Rep		5.19 Rep		8.64 Rep		14.40 Rep		24.00 Rep	
	1	2	3	4	1	2	1	2	1	2	1	2	1	2	1	2
Severe																
Stunted																
Gut		1				2		1			1	3			2	3
Edema:																
Multiple											1					
Cardiac											1			3		
Abdominal											1					
Facial											1					
Cephalic																
Optic											1					
Tail																
Notochord						1				1		3	3		1	
Fin																
Face	1				1	1	1		1	1		3	3	4	3	3
Eye																
Brain																
Hemorrhage																
Cardiac																2
Blisters																
Other*						1						3	1	3	1	
# Malformed	1	1	0	0	1	2	1	1	1	2	1	5	4	7	5	3

* Anal edema.

Table A3-4. FETAX Toxicity Test Data - Embryonic Growth (Length) after 96 Hours Exposure to Chemical U2723 - Assay 3

Concentration (mg/L)	Rep	Number Embryos Alive	Average Length (mm)	Mean Average Length (mm)
Control	1	25	9.54	9.47
	2	25	9.64	
	3	25	9.31	
	4	25	9.40	
1.82	1	25	8.83	9.10
	2	25	9.36	
3.07	1	25	9.11	9.28
	2	25	9.44	
5.19	1	25	9.25	9.28
	2	25	9.30	
8.64	1	23	8.70	8.51*
	2	20	8.31	
14.40	1	14	8.08	8.11*
	2	14	8.14	
24.00	1	6	8.06	7.80*
	2	5	7.53	

* Significantly different at alpha = 0.05 (Bonferroni T-Test)

APPENDIX 4

ASSAY 1 RAW DATA SHEETS FOR FROG EMBRYO TERATOGENESIS ASSAY-*XENOPUS* (FETAX) AND 6-AMINONICOTINAMIDE REFERENCE TOXICANT FOR CHEMICAL U2723

Note: The nominal concentrations of 1.9, 3.2, 5.4, 9.0, 15.0 and 25.0 mg/L in the raw data correspond to the nominal concentrations of 1.82, 3.07, 5.19, 8.64, 14.40 and 24.00 mg a.i./L in the report.

FORM 8D (Page 1 ~~of 2~~): FETAX TOTAL SUMMARY DATA SHEET

Notebook/Binder No. ______
Page ______

Test Number Definitive Test #1
Investigator Steve Turley
Laboratory WMD-WISEC

Test Start Date 5/16/00
Test End Date 5/19/00

Test Material U2723
Source ______
Lot Number ______

Concentration Stock - 25.0 mg/L
Composition/Purity ______

Petri Dish Number	Concentration	Initial No. of Embryos	Total Mortality	% Mortality	Mean % Mortality	Total No. of Embryos Alive at 96 hrs.	Total No. Malformed	% Malformed	Mean % Malformed	Average Length (mm)	Mean Average Length (mm)
1	Control A	25	1	4	1	24	1	4.2	4.0	9.05	8.59
2	Control B	25	0	0		25	1	4.0		8.40	
3	Control C	25	0	0		25	1	4.0		8.29	
4	Control D	25	0	0		25	1	4.0		8.62	
5	1.9 mg/L A	25	1	4	2	24	2	8.3	8.2	8.44	8.29
6	1.9 mg/L B	25	0	0		25	2	8.0		8.14	
7	3.2 mg/L A	25	1	4	4	24	2	8.3	14.6	8.88	8.80
8	3.2 mg/L B	25	1	4		24	5	20.8		8.72	
9	5.4 mg/L A	25	2	8	10	23	7	30.4	22.0	8.42	8.51
10	5.4 mg/L B	25	3	12		22	3	13.6		8.60	
11	9.0 mg/L A	25	4	16	12	21	4	19.0	24.7	8.83	8.71
12	9.0 mg/L B	25	2	8		23	7	30.4		8.58	
13	15.0 mg/L A	25	9	36	38	16	9	56.3	64.8	8.41	8.08
14	15.0 mg/L B	25	10	40		15	11	73.3		7.74	
15	25.0 mg/L A	25	25	100	100	0					
16	25.0 mg/L B	25	25	100		0					
17											
18	6 Aminonicotinamide										
19	5.5 mg/L A	25	2	8	10%	23	15	65.2	55.4	8.19	8.17
20	5.5 mg/L B	25	3	12		22	10	45.5		8.15	
21	2500 mg/L A	25	25	100	100	0					
22	2500 mg/L B	25	25	100		0					
23											
24											

Prepared By Steve D Turley Date 5/25/00

Reviewed By ______ Date ______

FORM 8C: FETAX MORTALITY DATA

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Test Number Definitive Test 1
Test Material U2723

Petri Dish/ Aquarium ID	Concentration	Initial No. of Embryos	Mortality 24 Hr.	48 Hr.	72 Hr.	96 Hr.	Total No. Dead Embryos	% Dead Embryos	Total No. Embryos Alive/Fixed
01	FETAX Control A	25	0	0	0	1	1	4	24
02	FETAX Control B	25	0	0	0	0	0	0	25
03	FETAX Control C	25	0	0	0	0	0	0	25
04	FETAX Control D	25	0	0	0	0	0	0	25
05	1.9 mg/L A	25	0	0	1	0	1	4	24
06	1.9 mg/L B	25	0	0	0	0	0	0	25
07	3.2 mg/L A	25	0	0	0	1	1	4	24
08	3.2 mg/L B	25	0	0	0	1	1	4	24
09	5.4 mg/L A	25	0	2	0	0	2	8	23
10	5.4 mg/L B	25	0	0	2	1	3	12	22
11	9.0 mg/L A	25	2	2	0	0	4	16	21
12	9.0 mg/L B	25	0	2	0	0	2	8	23
13	15.0 mg/L A	25	0	2	7	0	9	36	16
14	15.0 mg/L B	25	2	1	5	2	10	40	15
15	25.0 mg/L A	25	0	2	19	4	25	100	0
16	25.0 mg/L B	25	0	6	17	2	25	100	0
		By ST Date 5/15	By ST Date 5/16	By ST Date 5/17	By ST Date 5/18	By ST Date 5/19			By ST Date 5/19

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FORM 8H: FETAX INDIVIDUAL EMBRYO MALFORMATION DATA

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Petri Dish (Vial) No. 01 Test Number DeSimone Test #1 Data Collected By Stew Turley

Concentration FETAX Control A Test Material U2723 Date 5/15-6/19/00 No. Fixed Embryos 24

EMBRYO NO.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	TOTAL
NOT REMARKABLE	X	X		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X		23
MALFORMED			X																							1
MALFORMATIONS:																										
Severe																										
Stunted																										
Cut				X																						
Edema																										
Multiple																										
Cardiac																										
Abdominal																										
Facial																										
Cephalic																										
Optic																										
Tail																										
Notochord																										
Fin																										
Face				X																						
Eye																										
Brain																										
Hemorrhage																										
Cardiac																										
Blisters																										
Other-Specify																										

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Petri Dish (Vial) No. 02 Test Number Definitive Test #1 Date Collected By Steve Turley
Concentration FETAX Control B Test Material U2723 Date 5/16-6/1/00 No. Fixed Embryos 25

EMBRYO NO.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	TOTAL
NOT REMARKABLE	X	X	X	X		X	F	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	24
MALFORMED					X																					1
MALFORMATIONS:																										
Severe																										
Stunted																										
Cut					X																					
Edema																										
Multiple																										
Cardiac					X																					
Abdominal																										
Facial																										
Cephalic																										
Optic																										
Tail																										
Notochord																										
Fin																										
Face																										
Eye																										
Brain																										
Hemorrhage																										
Cardiac																										
Blisters																										
Other-Specify																										

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Petri Dish (Vial) No. 03 Test Number Definitive Test # 1 Data Collected By Steve Turley
Concentration FETAX Control C Test Material U2723 Date 5/15-5/19/00 No. Fixed Embryos 25

EMBRYO NO.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	TOTAL
NOT REMARKABLE		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	24
MALFORMED	X																									1
MALFORMATIONS:																										
Severe																										
Stunted																										
Cvd	X																									
Edema																										
Multiple																										
Cardiac																										
Abdominal																										
Facial																										
Cephalic																										
Optic																										
Tail																										
Notochord																										
Fin																										
Face																										
Eye																										
Brain																										
Hemorrhage																										
Cardiac																										
Blisters																										
Other-Specify																										

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Petri Dish (Vial) No. 04 Test Number Definitive Test #1 Data Collected By Steve Turley

Concentration FETAX Control D Test Material 112723 Date 5/15-5/19/00 No. Fixed Embryos 25

EMBRYO NO.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	TOTAL
NOT REMARKABLE		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	24
MALFORMED	X																									1
MALFORMATIONS:																										
Severe																										
Stunted																										
Gut	X																									
Edema																										
Multiple																										
Cardiac																										
Abdominal																										
Facial																										
Cephalic																										
Optic																										
Tail																										
Notochord																										
Fin																										
Face	X																									
Eye																										
Brain																										
Hemorrhage																										
Cardiac																										
Blisters																										
Other-Specify																										

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Petri Dish (Mab) No. 05 Test Number Definitive Test # 1 Data Collected By Steve Torley
Concentration 1.9 mg/L A Test Material U2723 Date 5/16-5/19/20 No. Fixed Embryos 24

EMBRYO NO.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	TOTAL
NOT REMARKABLE			X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X		22
MALFORMED	X	X																								2
MALFORMATIONS:																										
Severe																										
Stunted																										
Cut	X																									
Edema																										
Multiple																										
Cardiac																										
Abdominal																										
Facial																										
Cephalic																										
Optic																										
Tail																										
Notochord																										
Fin																										
Face																										
Eye	X	X																								
Brain																										
Hemorrhage																										
Cardiac																										
Blisters																										
Other-Specify																										

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Petri Dish (Vial) No. 96 Test Number DeSoto Tax #1 Data Collected By Steve Turley
Concentration 1.2 mg/L B Test Material U2723 Date 5/16-6/1/00 No. Fixed Embryos 25

EMBRYO NO.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	TOTAL
NOT REMEASURABLE			X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	23
MALFORMED	X	X																								2
MALFORMATIONS:																										
Severe																										
Stunted																										
Gut		X																								
Edema																										
Multiple																										
Cardiac																										
Abdominal																										
Facial																										
Cephalic																										
Optic																										
Tail																										
Notochord																										
Fin																										
Face																										
Eye																										
Brain																										
Hemorrhage																										
Cardiac																										
Blisters																										
Other-Specify																										

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Petri Dish (Vial) No. 07 Test Number Definitive Test 1 Data Collected By Steve Turley

Concentration 3.2 mg/L A Test Material U2723 Date 5/15-5/19/00 No. Fixed Embryos 24

EMBRYO NO.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	TOTAL
NOT REMARKABLE			X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X		22
MALFORMED	X	X																								2
MALFORMATIONS:																										
Severe																										
Stunted																										
Gut	X	X																								
Edema																										
Multiple																										
Cardiac		X																								
Abdominal		X																								
Facial																										
Cephalic																										
Optic																										
Tail																										
Notochord																										
Fin																										
Face		X																								
Eye																										
Brain																										
Hemorrhage																										
Cardiac																										
Blisters																										
Other-Specify																										

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Petri Dish (Vial) No. 08 Test Number Definitive Test 1 Data Collected By Steve Tuley

Concentration 3.2 mg/L B Test Material U2723 Date 5/15-5/19/00 No. Fixed Embryos 24

EMBRYO NO.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	TOTAL
NOT REMARKABLE						X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X		19
MALFORMED	X	X	X	X	X																					5
MALFORMATIONS:																										
Severe																										
Stunted																										
Gut	X	X	X	X	X																					
Edema																										
Multiple			X		X																					
Cardiac		X	X		X																					
Abdominal	X		X		X																					
Facial			X		X																					
Cephalic																										
Optic			X		X																					
Tail																										
Notochord																										
Fin																										
Face		X																								
Eye																										
Brain																										
Hemorrhage																										
Cardiac																										
Blisters																										
Other-Specify																										

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Petri Dish (Vial) No. 09 Test Number Definitive Test 1 Data Collected By Steve Tuley
Concentration 5.4 mg/L A Test Material 02723 Date 5/15/1-5/19/00 No. Fixed Embryos 23

EMBRYO NO.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	TOTAL
NOT REMARKABLE								X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X			16
MALFORMED	X	X	X	X	X	X	X																			7
MALFORMATIONS:																										
Severe																										
Stunted																										
Cut		X	X	X	X	X	X	X																		
Edema																										
Multiple		X			X																					
Cardiac					X																					
Abdominal																										
Facial					X																					
Cephalic																										
Optic		X			X																					
Tail																										
Notochord																										
Fin																										
Face		X	X	X			X																			
Eye																										
Brain																										
Hemorrhage																										
Cardiac			X	X																						
Blisters																										
Other-Specify																										

Reviewed By_____ Date_____

FORM 8H: FETAX INDIVIDUAL EMBRYO MALFORMATION DATA

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Petri Dish (Vial) No. 10 Test Number Definitive Test 1
Concentration 54 mg/L B Test Material U2723
Date 5/15-5/19/00 Data Collected By Steve Turley
No. Fixed Embryos 22

EMBRYO NO.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	TOTAL
NOT REMARKABLE					X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X				19
MALFORMED	X	X	X																							3
MALFORMATIONS:																										
Severe																										
Stunted																										
Cut	X	X	X																							
Edema																										
Multiple																										
Cardiac																										
Abdominal																										
Facial																										
Cephalic																										
Optic																										
Tail																										
Notochord																										
Fin																										
Face			X	X																						
Eye																										
Brain																										
Hemorrhage																										
Cardiac																										
Blisters																										
Other-Specify																										

Reviewed By _____ Date _____

FORM 8H: FETAX INDIVIDUAL EMBRYO MALFORMATION DATA

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Petri Dish (Vial) No. 11 Test Number Definitive Test 1 Data Collected By Steve Tedy
Concentration 2.0 mg/L A Test Material U2723 Date 5/15-5/19/00 No. Fixed Embryos 21

EMBRYO NO.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	TOTAL
NOT REMARKABLE					X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X			17
MALFORMED	X	X	X	X																						4
MALFORMATIONS:																										
Severe																										
Stunted																										
Gut		X	X	X																						
Edema																										
Multiple		X	X																							
Cardiac		X	X																							
Abdominal		X	X																							
Facial		X	X																							
Cephalic																										
Optic			X																							
Tail																										
Notochord																										
Fin																										
Face				X	X																					
Eye																										
Brain																										
Hemorrhage																										
Cardiac																										
Blisters																										
Other-Specify																										

Reviewed By _____ Date _____

FORM 8H: FETAX INDIVIDUAL EMBRYO MALFORMATION DATA

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Petri Dish (Vial) No. 12 Test Number Defnate Test 1 Data Collected By Steve Turley
Concentration 2.0 mg/L AB Test Material UZ723 Date 5-15-5-19-90 No. Fixed Embryos 23

EMBRYO NO.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	TOTAL
NOT REMARKABLE								X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X			16
MALFORMED	X	X	X	X	X	X	X																			7
MALFORMATIONS:																										
Severe	X																									
Stunted																										
Coil	X	X	X		X	X																				
Edema	X																									
Multiple	X			X																						
Cardiac	X			X																						
Abdominal	X			X																						
Facial	X			X																						
Cephalic																										
Optic		X																								
Tail																										
Notochord	X																									
Fin																										
Face	X	X					X																			
Eye	X																									
Brain																										
Hemorrhage																										
Cardiac																										
Blisters																										
Other-Specify																										

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FORM 8H: FETAX INDIVIDUAL EMBRYO MALFORMATION DATA

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Petri Dish (Vial) No. 13 Test Number Dechlor. Tap 1 Data Collected By Steve Tuckw
Concentration 150 mg/L A Test Material 02723 Date 5/15-5/19/00 No. Fixed Embryos 16

EMBRYO NO.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	TOTAL
NOT REMARKABLE																										7
MALFORMED	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X										9
MALFORMATIONS:																										
Severe																										
Stunted																										
Cist		X	X	X	X		X		X	X																
Edema																										
Multiple							X	X	X	X																
Cardiac							X	X	X	X																
Abdominal							X	X	X																	
Facial							X	X	X	X																
Cephalic																										
Optic							X		X																	
Tail																										
Notochord									X																	
Fin																										
Face		X	X		X																					
Eye									X																	
Brain																										
Hemorrhage																										
Cardiac		X	X						X																	
Blisters																										
Other-Specify																										

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Petri Dish (Well) No. 14 Test Number Definitive Test 1 Data Collected By Steve Tuckey
Concentration 15.0 mg/l B Test Material U2723 Date 5/15-5/19/00 No. Fixed Embryos 15

EMBRYO NO.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	TOTAL
NOT REMARKABLE												X	X	X	X											4
MALFORMED	X	X	X	X	X	X	X	X	X	X	X															11
MALFORMATIONS:																										
Severe																										
Stunted																										
Cut	X	X	X	X	X	X	X	X	X	X	X															
Edema																										
Multiple	X			X				X																		
Cardiac	X																									
Abdominal	X							X																		
Facial	X			X																						
Cephalic																										
Optic	X	X	X	X	X			X																		
Tail																										
Notochord																										
Fin																										
Face	X	X	X	X	X			X																		
Eye																										
Brain																										
Hemorrhage																										
Cardiac			X																							
Blisters																										
Other-Specify																										

Reviewed By_____ Date_____

96 Hour Test Data

Effluent/Groundwater (Toxicant) Chemical U2723 Date: May 15-19, 2000

Definitive Test #1 Analyst: Steve Turley

Species: Xenopus laevis Age at Start of Test: Blastula (Stage 8-11)

Control	Day 0	Day 1		Day 2		Day 3		Day 4
FETAX Control	0 H	Old	New	Old	New	Old	New	Old
Temperature (°C)	24.0	24.0	24.0	24.0	24.0	24.0	24.0	24.0
D.O. (mg/L)	8.3	8.0	8.4	7.7	8.3	7.4	8.3	7.7
pH	7.74	7.26	7.24	7.16	7.22	7.11	7.13	7.11
Conductivity								
Alkalinity								
Hardness								

1.9 mg/L	Day 0	Day 1		Day 2		Day 3		Day 4
U2723	0 H	Old	New	Old	New	Old	New	Old
Temperature (°C)	24.0	24.0	24.0	24.0	24.0	24.0	24.0	24.0
D.O. (mg/L)	8.4	7.8	8.4	7.7	8.3	7.5	8.2	7.7
pH	7.78	7.24	7.45	7.17	7.25	7.13	7.20	7.15
Conductivity								
Alkalinity								
Hardness								

3.2 mg/L	Day 0	Day 1		Day 2		Day 3		Day 4
U2723	0 H	Old	New	Old	New	Old	New	Old
Temperature (°C)	24.0	24.0	24.0	24.0	24.0	24.0	24.0	24.0
D.O. (mg/L)	8.4	7.9	8.5	7.6	8.4	7.7	8.1	7.6
pH	7.79	7.30	7.38	7.20	7.28	7.16	7.23	7.18
Conductivity								
Alkalinity								
Hardness								

Definitive Test 1

96 Hour Test Data
Page 2 of 3

5.4 mg/L	Day 0	Day 1		Day 2		Day 3		Day 4
U2723	0 H	Old	New	Old	New	Old	New	Old
Temperature (°C)	24.0	24.0	24.0	24.0	24.0	24.0	24.0	24.0
D.O. (mg/L)	8.4	7.9	8.4	7.7	8.3	7.7	8.2	7.5
pH	7.78	7.34	7.43	7.23	7.32	7.17	7.25	7.19
Conductivity								
Alkalinity								
Hardness								

9.0 mg/L	Day 0	Day 1		Day 2		Day 3		Day 4
U2723	0 H	Old	New	Old	New	Old	New	Old
Temperature (°C)	24.0	24.0	24.0	24.0	24.0	24.0	24.0	24.0
D.O. (mg/L)	8.4	8.0	8.5	7.8	8.2	7.8	8.1	7.7
pH	7.78	7.35	7.43	7.23	7.34	7.25	7.26	7.18
Conductivity								
Alkalinity								
Hardness								

15.0 mg/L	Day 0	Day 1		Day 2		Day 3		Day 4
U2723	0 H	Old	New	Old	New	Old	New	Old
Temperature (°C)	24.0	24.0	24.0	24.0	24.0	24.0	24.0	24.0
D.O. (mg/L)	8.4	8.0	8.5	7.8	8.3	7.9	8.1	7.7
pH	7.76	7.34	7.44	7.23	7.35	7.18	7.26	7.20
Conductivity								
Alkalinity								
Hardness								

96 Hour Test Data
Page 3 of 3

25.0 mg/L	Day 0	Day 1		Day 2		Day 3		Day 4
	0 H	Old	New	Old	New	Old	New	Old
Temperature (°C)	24.0	24.0	24.0	24.0	24.0	24.0	24.0	24.0
D.O. (mg/L)	8.5	7.9	8.4	7.8	8.3	7.9	8.1	7.7
pH	7.62	7.33	7.32	7.25	7.27	6.95	7.18	7.19
Conductivity								
Alkalinity								
Hardness								

_____	Day 0	Day 1		Day 2		Day 3		Day 4
	0 H	Old	New	Old	New	Old	New	Old
Temperature (°C)								
D.O. (mg/L)								
pH								
Conductivity								
Alkalinity								
Hardness								

_____	Day 0	Day 1		Day 2		Day 3		Day 4
	0 H	Old	New	Old	New	Old	New	Old
Temperature (°C)								
D.O. (mg/L)								
pH								
Conductivity								
Alkalinity								
Hardness								

96 Hour Test Data

Effluent/Groundwater/Toxicant: 6-Aminouracil (Test 1) Date: 5/15/-5/19/00

Analyst: ST

Species: Xenopus laevis Age at Start of Test: Day 8 - Stage 51

~~Control~~	Day 0	Day 1		Day 2		Day 3		Day 4
55 mg/L (6-AN)	0 H	Old	New	Old	New	Old	New	Old
Temperature (°C)	24.0	24.0	24.0	24.0	24.0	24.0	24.0	24.0
D.O. (mg/L)	8.4	8.0	8.3	7.9	8.3	7.8	8.3	7.6
pH	7.56	7.31	7.27	7.19	7.30	7.16	7.26	7.07
Conductivity								
Alkalinity								
Hardness								

2500 mg/L (6-AN)	Day 0	Day 1		Day 2		Day 3		Day 4
	0 H	Old	New	Old	New	Old	New	Old
Temperature (°C)	24.0	24.0	24.0	24.0	24.0	24.0	24.0	24.0
D.O. (mg/L)	8.3	7.9	8.3	7.7	8.3	7.6	8.3	7.4
pH	7.44	7.26	7.20	7.14	7.20	7.10	7.17	7.04
Conductivity								
Alkalinity								
Hardness								

_____	Day 0	Day 1		Day 2		Day 3		Day 4
	0 H	Old	New	Old	New	Old	New	Old
Temperature (°C)								
D.O. (mg/L)								
pH								
Conductivity								
Alkalinity								
Hardness								

Chemical U2723- Definitive Test 1
Embryo Length Measurements (mm)
Page 1 of x 3

	Control A	Control B	Control C	Control D	1.9 mg/L A	1.9 mg/L B
1	8.86	8.66	8.96	8.78	8.76	8.48
2	9.06	8.03	8.83	8.91	9	6.26
3	9.36	8.25	8.83	8.01	9.11	8.23
4	9.25	8.69	8.07	8.63	8.25	8.2
5	8.93	8.1	8.44	8.08	8.5	8.75
6	9.32	8.35	9.41	8.61	8.82	8.31
7	9.76	8.4	8.05	8.83	7.44	8.3
8	9.29	7.74	7.51	9.1	8.3	7.83
9	9.37	8.38	7.88	8.94	8.38	7.96
10	9.54	8.68	7.96	9	8.23	8.53
11	9.39	7.29	8.1	8.83	8.93	8.16
12	9.28	8.28	7.96	7.58	8.58	7.27
13	9.29	8.58	9.05	8.01	7.59	8.5
14	8.76	8.81	8.17	8.33	9.45	8.75
15	8.22	8.36	8.28	8.75	8.51	8.41
16	8.19	8.6	7.45	8.83	8.87	8.25
17	7.93	8.85	7.94	8.4	8.17	8.16
18	9.56	8.72	8.17	8.14	8.77	8.16
19	8.96	8.36	7.34	8.54	8.24	8.03
20	8.51	8.55	10.06	8.78	7.88	8.47
21	9.17	9.02	9.22	8.95	9.05	8.21
22	9.19	8.16	7.47	8.53	8.47	8.27
23	8.58	7.87	7.81	9.46	7.93	7.67
24	9.45	8.73	8.02	8.29	7.42	8.55
25		8.64	8.36	9.12		7.67
Mean	9.0508333	8.404	8.2936	8.6172	8.44375	8.1352
Min	7.93	7.29	7.34	7.58	7.42	6.26
Max	9.76	9.02	10.06	9.46	9.45	8.75
Stand. Dev.	0.4723477	0.3886194	0.6695949	0.429627	0.5323027	0.5190466

Chemical U2723- Definitive Test 1
Embryo Length Measurements (mm)
Page 2 of x 3

	3.2 mg/L A	3.2 mg/L B	5.4 mg/L A	5.4 mg/L B	9.0 mg/L A	9.0 mg/L B
1	9.24	9.22	5.61	9.7	8.73	9.16
2	9.15	8.66	8.41	8.92	8.89	8.52
3	8.84	9.4	6.85	7.33	7.24	9.18
4	7.53	8.78	6.63	9.14	8.4	6.34
5	8.95	8.74	8.59	8.98	8.37	8.6
6	9.21	8.76	7.42	8.25	8.44	8.84
7	8.01	9.09	8.98	8.45	9.51	8.89
8	9.25	8.34	8.73	8.37	9.23	7.41
9	9.54	9.37	8.92	6.25	8.66	8.83
10	9.39	8.44	8.6	8.84	8.87	8.73
11	8.07	8.35	8.39	8.75	6.98	9.26
12	10.08	8.03	7.61	9.26	8.32	9.45
13	9.2	9.04	8.51	7.97	8.83	9.42
14	8.84	9.18	9.35	8.79	9.15	9.22
15	8.94	8.76	8.16	8.5	9.5	8.99
16	8.09	8.07	9.1	8.93	9.45	9.01
17	9.38	7.17	9.41	9.11	9.81	9.82
18	8.78	8.97	8.72	9.76	9.09	9.4
19	8.08	9.18	8.91	8.72	8.94	6.95
20	9.66	9.58	9.32	8.33	9.6	7.57
21	8.84	8.27	9.57	8.58	9.52	8.24
22	9.28	8.61	8.79	8.27		9.24
23	7.69	8.23	9			6.33
24	9.16	9.04				
25						
Mean	8.8833333	8.72	8.4165217	8.6	8.8347619	8.5826087
Min	7.53	7.17	5.61	6.25	6.98	6.33
Max	10.08	9.58	9.57	9.76	9.81	9.82
Stand. Dev.	0.6501483	0.5448654	0.9830824	0.7544029	0.7235373	0.9891145

Chemical U2723- Definitive Test 1
Embryo Length Measurements (mm)
Page 3 of 3

	15 mg/L A	15 mg/L B	25 mg/L A	25 mg/L B	6-Aminonicotinamide 5.5 mg/L A	5.5 mg/L B
1	9	6.55			7.95	6.99
2	9.57	7.85			8.21	7.77
3	7.8	7.65			8.46	7.99
4	7.73	7.76			8.3	8.92
5	7.79	8.9			9.6	8.4
6	9.5	7.28			9.02	7.8
7	7.5	7.43			7.46	9.12
8	8.86	8.52			7.59	9.23
9	7.46	8.2			7.7	8.11
10	9.28	8.48			8.1	7.55
11	7.84	6.51			8.16	8.57
12	10.24				8.01	7.96
13	8.51				7.99	8.41
14	7.14				9.11	8.69
15	8.91				8.81	7.95
16	7.41				8.41	8.12
17					8.16	9.04
18					7.44	8.27
19					7.39	7.84
20					7.8	7.93
21					8.12	7.15
22					8.91	7.38
23					7.65	
24						
25						
Mean	8.40875	7.7390909			8.1891304	8.145
Min	7.14	6.51			7.39	6.99
Max	10.24	8.9			9.6	9.23
Stand. Dev.	0.9429519	0.7726895			0.5832504	0.6140556

FORM 8H: FETAX INDIVIDUAL EMBRYO MALFORMATION DATA

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Petri Dish (Vial) No. 19 Test Number Definitive Test 1 Data Collected By Steve Turley
Concentration 5.5mg/L A Test Material 6-Aminonicotinamide Date 5/15-5/19/17 No. Fixed Embryos 23

EMBRYO NO.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	TOTAL
NOT REMARKABLE																X	X	X	X	X	X	X	X			8
MALFORMED	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X											15
MALFORMATIONS:																										
Severe																										
Stunted																										
Gut	X	X	X	X	X	X	X		X	X		X	X	X												
Edema																										
Multiple								X		X																
Cardiac								X		X																
Abdominal				X			X	X		X			X													
Facial							X	X																		
Cephalic								X																		
Optic																										
Tail																										
Notochord	X		X								X	X			X											
Fin																										
Face			X		X	X	X				X	X		X												
Eye																										
Brain																										
Hemorrhage																										
Cardiac																										
Blisters			X						X																	
Other-Specify																										

Reviewed By_____ Date_____

FORM 8H: FETAX INDIVIDUAL EMBRYO MALFORMATION DATA

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Petri Dish (Vial) No. 20 Test Number Definitive Test 1 Data Collected By Stac Turley
Concentration 5.5 mg/L B Test Material 6-Aminonicotinamide Date 5/15-5/19/00 No. Fixed Embryos 22

EMBRYO NO.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	TOTAL
NOT REMARKABLE											X	X	X	X	X	X	X	X	X	X	X	X	—	—	—	12
MALFORMED	X	X	X	X	X	X	X	X	X	X																10
MALFORMATIONS:																										
Severe																										
Stunted																										
Gut			X	X	X	X		X		X																
Edema																										
Multiple	X			X				X																		
Cardiac	X			X				X																		
Abdominal	X		X	X				X																		
Facial	X							X																		
Cephalic																										
Optic	X							X																		
Tail																										
Notochord		X					X			X																
Fin																										
Face		X			X	X	X		X	X																
Eye																										
Brain																										
Hemorrhage																										
Cardiac																										
Blisters							X																			
Other-Specify																										

Reviewed By__________________ Date______

TRIMMED SPEARMAN-KARBER METHOD. VERSION 1.5

U2723 - Assay 1 Mortality - LC50

DATE: 5/15/00 TEST NUMBER: 1 DURATION: 96 h
TOXICANT : u2723
SPECIES: xenopus

RAW DATA: ---	Concentration (mg/l)	Number Exposed	Mortalities
-	.00	100	1
	2.00	50	1
	2.83	50	2
	4.73	50	5
	7.90	50	6
	14.70	50	19
	24.60	50	50

SPEARMAN-KARBER TRIM: 1.01%

SPEARMAN-KARBER ESTIMATES: LC50: 13.77
95% LOWER CONFIDENCE: 12.38
95% UPPER CONFIDENCE: 15.31

NOTE: MORTALITY PROPORTIONS WERE NOT MONOTONICALLY INCREASING.
ADJUSTMENTS WERE MADE PRIOR TO SPEARMAN-KARBER ESTIMATION.

TRIMMED SPEARMAN-KARBER METHOD. VERSION 1.5

U2723 - Assay 1 - Malformations (EC50)

DATE: 5/15/00 TEST NUMBER: 1 DURATION: 96 h
TOXICANT : U2723
SPECIES: xenopus

RAW DATA: Concentration (mg/l)	Number Exposed	Mortalities
.00	99	4
2.00	49	4
2.83	48	7
4.73	45	10
7.90	44	11
14.70	31	20

SPEARMAN-KARBER TRIM: 36.98%

SPEARMAN-KARBER ESTIMATES: EC50: 12.08
95% LOWER CONFIDENCE: 10.00
95% UPPER CONFIDENCE: 14.58

NOTE: MORTALITY PROPORTIONS WERE NOT MONOTONICALLY INCREASING.
ADJUSTMENTS WERE MADE PRIOR TO SPEARMAN-KARBER ESTIMATION.

TI = LC50/EC50 = 13.77/12.08 = 1.14

Title: u2723:Assay 1: embryo length data (mg)
File: ASSAY1LE. Transform: NO TRANSFORMATION

Shapiro - Wilk's Test for Normality

D = 0.6683
W = 0.9649

Critical W = 0.8250 (alpha = 0.01 , N = 14)
W = 0.8740 (alpha = 0.05 , N = 14)

Data PASS normality test (alpha = 0.01). Continue analysis.

Title: u2723:Assay 1: embryo length data (mg)
File: ASSAY1LE. Transform: NO TRANSFORMATION

Bartlett's Test for Homogeneity of Variance

Calculated B1 statistic = 2.3953 (p-value = 0.7922)

Data PASS B1 homogeneity test at 0.01 level. Continue analysis.

Critical B = 15.0863 (alpha = 0.01, df = 5)
= 11.0705 (alpha = 0.05, df = 5)

Using Average Degrees of Freedom
(Based on average replicate size of 2.33)

Calculated B2 statistic = 2.2190 (p-value = 0.8181)

Data PASS B2 homogeneity test at 0.01 level. Continue analysis.

Title: u2723:Assay 1: embryo length data (mg)
File: ASSAY1LE. Transform: NO TRANSFORMATION

ANOVA Table

SOURCE	DF	SS	MS	F
Between	5	0.7451	0.1490	1.7838
Within (Error)	8	0.6683	0.0835	
Total	13	1.4134		

(p-value = 0.2224)

Critical F = 6.6318 (alpha = 0.01, df = 5,8)
= 3.6875 (alpha = 0.05, df = 5,8)

Since F < Critical F FAIL TO REJECT Ho: All equal (alpha = 0.05)

Title: u2723:Assay 1: embryo length data (mg)
File: ASSAY1LE. Transform: NO TRANSFORMATION

Bonferroni t-Test - TABLE 1 OF 2 Ho: Control<Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	t STAT	SIG 0.05
1	con	8.5900	8.5900		
2	2.00 mg/l	8.2900	8.2900	1.1985	
3	2.83 mg/l	8.8000	8.8000	-0.8390	
4	4.73 mg/l	8.5100	8.5100	0.3196	
5	7.90 mg/l	8.7050	8.7050	-0.4594	
6	14.70 mg/l	8.0750	8.0750	2.0575	

Bonferroni t critical value = 2.8965 (1 Tailed, alpha = 0.05, df = 5,8)

Title: u2723:Assay 1: embryo length data (mg)
File: ASSAY1LE. Transform: NO TRANSFORMATION

Bonferroni t-Test - TABLE 2 OF 2 Ho: Control<Treatment

GROUP	IDENTIFICATION	NUM OF REPS	MIN SIG DIFF (IN ORIG. UNITS)	% OF CONTROL	DIFFERENCE FROM CONTROL
1	con	4			
2	2.00 mg/l	2	0.7250	8.4	0.3000
3	2.83 mg/l	2	0.7250	8.4	-0.2100
4	4.73 mg/l	2	0.7250	8.4	0.0800
5	7.90 mg/l	2	0.7250	8.4	-0.1150
6	14.70 mg/l	2	0.7250	8.4	0.5150

APPENDIX 5

ASSAY 2 RAW DATA SHEETS FOR FROG EMBRYO TERATOGENESIS ASSAY-*XENOPUS* (FETAX) AND 6-AMINONICOTINAMIDE REFERENCE TOXICANT FOR CHEMICAL U2723

Note: The nominal concentrations of 1.9, 3.2, 5.4, 9.0, 15.0 and 25.0 mg/L in the raw data correspond to the nominal concentrations of 1.82, 3.07, 5.19, 8.64, 14.40 and 24.00 mg a.i./L in the report.

FORM 8D (Page 1 ~~of 2~~): FETAX TOTAL SUMMARY DATA SHEET Notebook/Binder No.____ Page____

Test Number 2 Test Start Date 5/22/00 Test Material U2723 Concentration____
Investigator Sue Lacy Test End Date 5/26/00 Source____ Composition/Purity____
Laboratory WIL-WISEC Lot Number____

Petri Dish Number	Concentration	Initial No. of Embryos	Total Mortality	% Mortality	Mean % Mortality	Total No. of Embryos Alive at 96 hrs.	Total No. Malformed	% Malformed	Mean % Malformed	Average Length	Mean Average Length
1	FETAX Cntl A	25	0	0	1.0	25	1	4.0	4.1	8.94	8.88
2	FETAX Cntl B	25	0	0		25	2	8.0		8.81	
3	FETAX Cntl C	25	0	0		25	0	0		8.90	
4	FETAX Cntl D	25	1	4		24	1	4.2		8.88	
5	1.9 mg/L A	25	0	0	0	25	2	8.0	8.0	8.39	8.45
6	1.9 mg/L B	25	0	0		25	2	8.0		8.50	
7	3.2 mg/L A	25	2	8	10.0	23	2	8.7	4.4	8.42	8.58
8	3.2 mg/L B	25	3	12		22	0	0		8.71	
9	5.4 mg/L A	25	1	4	8.0	24	1	4.2	11.2	8.69	8.72
10	5.4 mg/L B	25	3	12		22	4	18.2		8.74	
11	9.0 mg/L A	25	3	12	10.0	22	4	18.2	20.0	7.94	7.93
11	9.0 mg/L B	25	2	8		23	5	21.7		7.92	
11	15.0 mg/L A	25	3	12	30.0	22	4	18.2	43.7	7.89	7.51
11	15.0 mg/L B	25	12	48		13	9	69.2		7.13	
11	25.0 mg/L A	25	16	64	70.0	9	6	66.7	66.7	7.50	7.39
11	25.0 mg/L B	25	19	76		6	4	66.7		7.27	
11											
11	6-Ammediol										
11	55 mg/L A	25	1	4	6.0	24	12	50.0	55.1	7.41	7.35
11	55 mg/L B	25	2	8		23	14	60.1		7.28	
11	250 mg/L A	25	25	100	100	—					
11	280 mg/L B	25	25	100		—					
11											
11											

Reviewed By____ Date____ Prepared By____ Date____

FORM 8C: FETAX MORTALITY DATA

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Test Number Definitive Test 2

Test Material WI7723

Petri Dish/ Aquarium ID	Concentration	Initial No. of Embryos	Mortality 24 Hr.	48 Hr.	72 Hr.	96 Hr.	Total No. Dead Embryos	% Dead Embryos	Total No. Embryos Alive/Fixed
01	FETAX Control A	25	0	0	0	0	0	0	25
02	FETAX Control B	25	0	0	0	0	0	0	25
03	FETAX Control C	25	0	0	0	0	0	0	25
04	FETAX Control D	25	0	0	0	1	1	4.0	24
05	1.9 mg/L A	25	0	0	0	0	0	0	25
06	1.9 mg/L B	25	0	0	0	0	0	0	25
07	3.2 mg/L A	25	0	2	0	0	2	8	23
08	3.2 mg/L B	25	0	1	0	2	3	12	22
09	5.4 mg/L A	25	0	0	0	1	1	4	24
10	5.4 mg/L B	25	0	1	0	2	3	12	22
11	9.0 mg/L A	25	0	1	0	2	3	12	22
12	9.0 mg/L B	25	0	1	0	1	2	8	23
13	15.0 mg/L A	25	1	0	0	2	3	12	22
14	15.0 mg/L B	25	0	3	3	6	12	48	13
15	25.0 mg/L A	25	0	1	13	2	16	64	9
16	25.0 mg/L B	25	2	1	11	5	19	76	6
	6-Aminonicotinamide								
19	5.5 mg/L A	25	0	0	1	0	1	4	24
20	5.5 mg/L B	25	0	0	1	1	2	8	23
21	2500 mg/L A	25	0	9	8	8	25	100	0
22	2500 mg/L B	25	4	8	8	5	25	100	0
		By [illegible] Date 5/22	By ST Date 5/23	By ST Date 5/24	By ST Date 5/25	By ST Date 5/26			By ST Date 5/26

Reviewed By_____ Date_____

FORM 8H: FETAX INDIVIDUAL EMBRYO MALFORMATION DATA

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Petri Dish (Vial) No. 01 Test Number Definitive Test 2 Data Collected By Steve Turley
Concentration FETAX Control A Test Material U2723 Date 5/22-5/26/00 No. Fixed Embryos 25

EMBRYO NO.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	TOTAL
NOT REMARKABLE		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	24
MALFORMED	X																									1
MALFORMATIONS:																										
Severe																										
Stunted																										
Gut																										
Edema																										
Multiple																										
Cardiac																										
Abdominal																										
Facial																										
Cephalic																										
Optic																										
Tail																										
Notochord																										
Fin																										
Face																										
Eye																										
Brain																										
Hemorrhage																										
Cardiac																										
Blisters																										
Other-Specify																										

Reviewed By_____ Date_____

FORM 8H: FETAX INDIVIDUAL EMBRYO MALFORMATION DATA

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Petri Dish (Vial) No. 02 Test Number DeSanto Test 2 Data Collected By Steve Turley
Concentration FETAX Control B Test Material U2723 Date 5/22-5/26/00 No. Fixed Embryos 25

EMBRYO NO.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	TOTAL
NOT REMARKABLE			X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	23
MALFORMED		X	X																							2
MALFORMATIONS:																										
Severe																										
Stunted																										
Cvl		X																								
Edema																										
Multiple																										
Cardiac																										
Abdominal																										
Facial																										
Cephalic																										
Optic																										
Tail																										
Notochord																										
Fin																										
Face		X	X																							
Eye																										
Brain																										
Hemorrhage																										
Cardiac		X	X																							
Blisters																										
Other-Specify																										

Reviewed By _____ Date _____

FORM 8H: FETAX INDIVIDUAL EMBRYO MALFORMATION DATA

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Petri Dish (Vial) No. 03 Test Number DeSoto Test 2 Data Collected By Stru Turley
Concentration FETAX Control C Test Material U2723 Date 5/22-5/26/00 No. Fixed Embryos 25

EMBRYO NO.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	TOTAL
NOT REMARKABLE	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	25
MALFORMED																										0
MALFORMATIONS:																										
Severe																										
Shorted																										
Cut																										
Edema																										
Multiple																										
Cardiac																										
Abdominal																										
Facial																										
Cephalic																										
Optic																										
Tail																										
Notochord																										
Fin																										
Face																										
Eye																										
Brain																										
Hemorrhage																										
Cardiac																										
Blisters																										
Other-Specify																										

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Petri Dish (Vial) No. 04 Test Number DeSmike Test 2 Data Collected By Steve Turley
Concentration FETAX Control D Test Material U2723 Date 5/22-5/26/00 No. Fixed Embryos 24

EMBRYO NO.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	TOTAL
NOT REMARKABLE		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X		23
MALFORMED	X																									1
MALFORMATIONS:																										
Severe																										
Shorted																										
Gut																										
Edema																										
Multiple																										
Cardiac																										
Abdominal																										
Facial																										
Cephalic																										
Optic																										
Tail																										
Notochord																			X							
Fin																										
Face																										
Eye																										
Brain																										
Hemorrhage																										
Cardiac																										
Blisters																										
Other-Specify																										

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Petri Dish (Vial) No. 05 Test Number DeSoto Tst 2 Data Collected By Steve Turley
Concentration 1.9 mg/L A Test Material 02723 Date 5/22-5/26/00 No. Fixed Embryos 25

EMBRYO NO.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	TOTAL
NOT REMARKABLE			X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	23
MALFORMED	X	X																								2
MALFORMATIONS:																										
Severe																										
Stunted																										
Cut			X																							
Edema																										
Multiple			X	X																						
Cardiac			X	X																						
Abdominal			X	X																						
Facial			X	X																						
Cephalic			X	X																						
Optic			X	X																						
Tail																										
Notochord																										
Fin																										
Face																										
Eye																										
Brain																										
Hemorrhage																										
Cardiac																										
Blisters																										
Other-Specify																										

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Petri Dish (Vial) No. 06 Test Number Definitive Tst 2 Data Collected By Steve Turley
Concentration 1.9 mg/L B Test Material U2723 Date 5/22-5/26/00 No. Fixed Embryos 25

EMBRYO NO.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	TOTAL
NOT REMARKABLE			X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	23
MALFORMED	X	X																								2
MALFORMATIONS:																										
Severe																										
Stunted																										
Gut		X																								
Edema																										
Multiple																										
Cardiac																										
Abdominal																										
Facial																										
Cephalic																										
Optic																										
Tail																										
Notochord																										
Fin																										
Face																										
Eye																										
Brain																										
Hemorrhage																										
Cardiac																										
Blisters																										
Other-Specify																										

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Petri Dish (Vial) No. 07 Test Number DeSnake Test 2
Concentration 3.2 mg/L A Test Material U2723
Data Collected By Steve Turley
Date 5/22-5/26/00 No. Fixed Embryos 23

EMBRYO NO.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	TOTAL
NOT REMARKABLE			X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X		21
MALFORMED	X	X																								2
MALFORMATIONS:																										
Severe																										
Shorted																										
Cut		X																								
Edema																										
Multiple																										
Cardiac																										
Abdominal																										
Facial																										
Cephalic																										
Optic																										
Tail																										
Notochord		X	X																							
Fin																										
Face																										
Eye																										
Brain																										
Hemorrhage																										
Cardiac																										
Blisters																										
Other-Specify																										

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Petri Dish (Vial) No. 08 Test Number: Definitive Test 2 Data Collected By: Steve Tuley
Concentration 3.2 mg/L B Test Material: U2723 Date 5/22-5/26/00 No. Fixed Embryos 22

EMBRYO NO.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	TOTAL
NOT REMARKABLE	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X			22
MALFORMED																										0
MALFORMATIONS:																										
Severe																										
Stunted																										
Cut																										
Edema																										
Multiple																										
Cardiac																										
Abdominal																										
Facial																										
Cephalic																										
Optic																										
Tail																										
Notochord																										
Fin																										
Face																										
Eye																										
Brain																										
Hemorrhage																										
Cardiac																										
Blisters																										
Other-Specify																										

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Petri Dish (Vial) No. 09 Test Number Definitive Test 2 Data Collected By Steve Turley
Concentration 5.4 mg/L A Test Material U2723 Date 5/22-5/26/00 No. Fixed Embryos 24

EMBRYO NR.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	TOTAL
NOT REHABILABLE		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	23
MALFORMED	X																									1
MALFORMATIONS:																										
Severe																										
Stunted																										
Curl																										
Edema																										
Multiple																										
Cardiac																										
Abdominal																										
Facial																										
Cephalic	X																									
Optic																										
Tail																										
Notochord																										
Fin																										
Face																										
Eye																										
Brain																										
Hemorrhage																										
Cardiac																										
Blisters																										
Other-Specify																										

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Petri Dish (Vial) No. 10 Test Number Definitive Test 2 Data Collected By Steve Tinley

Concentration 5.4 mg/L B Test Material U2723 Date 5/22-5/26/00 No. Fixed Embryos 22

EMBRYO NO.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	TOTAL
NOT REMARKABLE					X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	—	—	—	18
MALFORMED	X	X	X	X																						4
MALFORMATIONS:																										
Severe																										
Stunted																										
Gut	X	X	X	X																						
Edema																										
Multiple	X		X																							
Cardiac	X		X																							
Abdominal	X		X	X																						
Facial	X		X	X																						
Cephalic																										
Optic	X																									
Tail																										
Notochord																										
Fin																										
Face		X	X																							
Eye																										
Brain																										
Hemorrhage																										
Cardiac																										
Blisters																										
Other-Specify																										

Reviewed By__________ Date__________

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Petri Dish (Vial) No. 11 Test Number Definitive Test 2 Data Collected By Steve Turley

Concentration 9.0 mg/L A Test Material U2723 Date 5/22-5/26/00 No. Fixed Embryos 22

EMBRYO NO.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	TOTAL
NOT REMARKABLE					X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X				18
MALFORMED	X	X	X	X																						4
MALFORMATIONS:																										
Severe																										
Stunted																										
Gut		X																								
Edema																										
Multiple																										
Cardiac	X		X																							
Abdominal																										
Facial																										
Cephalic																										
Optic																										
Tail																										
Notochord																										
Fin																										
Face	X			X																						
Eye																										
Brain																										
Hemorrhage																										
Cardiac																										
Blisters																										
Other-Specify			X																							

* And edema

Reviewed By______ Date______

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Petri Dish (Vial) No. 12 Test Number Definitive Test 2 Data Collected By Steve Turley
Concentration 2.0 mg/L B Test Material 012723 Date 5/22-5/26/00 No. Fixed Embryos 23

EMBRYO NO.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	TOTAL
NOT REMARKABLE						X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	—	—	18
MALFORMED	X	X	X	X	X																					5
MALFORMATIONS:																										
Severe																										
Stunted																										
Curl	X	X	X																							
Edema																										
Multiple	X																									
Cardiac	X																									
Abdominal	X																									
Facial	X																									
Cephalic																										
Optic																										
Tail																										
Notochord																										
Fin																										
Face			X	X	X																					
Eye																										
Brain																										
Hemorrhage																										
Cardiac																										
Blisters																										
Other-Specify																										

Reviewed By _____ Date _____

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Petri Dish (Vial) No. 013 Test Number Dilution Test 2 Data Collected By Steve Turley
Concentration 150 mM A Test Material 02723 Date 5/22-5/26/00 No. Fixed Embryos 22

EMBRYO NO.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	TOTAL
NOT REMARKABLE					X	X	A	M	X	X	X	X	X	X	X	X	X	X	X	X	X	X				18
MALFORMED	X	X	X	X																						4
MALFORMATIONS:																										
Severe																										
Stunted																										
Cut		X	X																							
Edema																										
Multiple																										
Cardiac																										
Abdominal				X																						
Facial																										
Cephalic																										
Optic																										
Tail																										
Notochord			X																							
Fin																										
Face																X										
Eye																										
Brain																										
Hemorrhage																										
Cardiac																										
Blisters																										
Other-Specify																										

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Petri Dish (Mat) No. 14 Test Number Definitive Test 2 Date Collected By Steve Tuley

Concentration 15.0 mg/L B Test Material U2723 Date 5/22/-5/26/00 No. Fixed Embryos 13

EMBRYO NO.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	TOTAL
NOT REMARKABLE										X	X	X	X													4
MALFORMED	X	X	X	X	X	X	X	X	X																	9
MALFORMATIONS:																										
Severe																										
Stunted																										
Cut		X	X	X			X	X	X																	
Edema																										
Multiple																										
Cardiac																										
Abdominal																										
Facial																										
Cephalic																										
Optic																										
Tail																										
Notochord	X																									
Fin																										
Face	X	X	X	X	X	X	X	X	X																	
Eye																										
Brain																										
Hemorrhage																										
Cardiac																										
Blisters																										
Other-Specify	X	X																								

* Anal edema

Reviewed By_____ Date_____

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Petri Dish (Vial) No. 15 Test Number Definitive Test 2
Concentration 250 mg/L A Test Material U2723
Data Collected By Steve Turley
Date 5/22 - 5/24/03 No. Fixed Embryos 9

EMBRYO NO.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	TOTAL
NOT REMARKABLE							X	X	X																	3
MALFORMED	X	X	X	X	X	X																				6
MALFORMATIONS:																										
Severe																										
Stunted																										
Cut	X	X	X	X	X	X																				
Edema																										
Multiple	X		X	X																						
Cardiac	X		X	X		X																				
Abdominal	X		X	X																						
Facial	X		X	X																						
Cephalic																										
Optic	X		X	X																						
Tail																										
Notochord																										
Fin																										
Face					X																					
Eye																										
Brain																										
Hemorrhage																										
Cardiac																										
Blisters																										
Other-Specify																										

Reviewed By_____ Date_____

FORM 8H: FETAX INDIVIDUAL EMBRYO MALFORMATION DATA

Notebook/Binder No.______

Page______

Petri Dish (Vial) No. 16 Test Number Definitive Test 2 Data Collected By Steve Tinley

Concentration 25.0 mg/L B Test Material U2723 Date 5/22-5/26/00 No. Fixed Embryos 6

EMBRYO NO.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	TOTAL
NOT REMARKABLE					X	X																				2
MALFORMED	X	X	X	X																						4
MALFORMATIONS:																										
Severe																										
Stunted																										
Gut	X	X	X	X																						
Edema																										
Multiple																										
Cardiac																										
Abdominal																										
Facial																										
Cephalic																										
Optic																										
Tail																										
Notochord																										
Fin																										
Face		X	X	X																						
Eye																										
Brain																										
Hemorrhage																										
Cardiac																										
Blisters																										
Other-Specify																										

Reviewed By______________________ Date__________

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96 Hour Test Data

Effluent/Groundwater/Toxicant: Chemical U2723 Date: 5/22-5/26/00

Definitive Test 2 Analyst: Steve Finley

Species: Xenopus laevis Age at Start of Test: Blastula (Stage 8-11

Control	Day 0	Day 1		Day 2		Day 3		Day 4
FETAX	0 H	Old	New	Old	New	Old	New	Old
Temperature (°C)	24.0	24.0	24.0	24.0	24.0	24.0	24.0	24.0
D.O. (mg/L)	8.2	8.3	8.2	7.6	8.1	7.5	8.1	7.6
pH	7.14	7.12	7.11	7.18	7.15	7.20	7.17	7.11
Conductivity								
Alkalinity								
Hardness								

1.9 mg/L	Day 0	Day 1		Day 2		Day 3		Day 4
U2723	0 H	Old	New	Old	New	Old	New	Old
Temperature (°C)	24.0	24.0	24.0	24.0	24.0	24.0	24.0	24.0
D.O. (mg/L)	8.2	8.3	8.2	7.8	8.1	7.6	8.2	7.6
pH	7.24	7.17	7.21	7.22	7.23	7.20	7.21	7.21
Conductivity								
Alkalinity								
Hardness								

3.2 mg/L	Day 0	Day 1		Day 2		Day 3		Day 4
U2723	0 H	Old	New	Old	New	Old	New	Old
Temperature (°C)	24.0	24.0	24.0	24.0	24.0	24.0	24.0	24.0
D.O. (mg/L)	8.1	8.0	8.2	7.5	8.0	7.5	8.1	7.5
pH	7.31	7.21	7.28	7.23	7.30	7.22	7.26	7.20
Conductivity								
Alkalinity								
Hardness								

Definitive Test 2

96 Hour Test Data
Page 2 of 3

5.4 mg/L	Day 0	Day 1		Day 2		Day 3		Day 4
U2723	0 H	Old	New	Old	New	Old	New	Old
Temperature (°C)	24.0	24.0	24.0	24.0	24.0	24.0	24.0	24.0
D.O. (mg/L)	8.2	8.1	8.2	7.2	8.1	7.6	8.1	7.5
pH	7.33	7.23	7.35	7.24	7.37	7.25	7.34	7.29
Conductivity								
Alkalinity								
Hardness								

9.0 mg/L	Day 0	Day 1		Day 2		Day 3		Day 4
U2723	0 H	Old	New	Old	New	Old	New	Old
Temperature (°C)	24.0	24.0	24.0	24.0	24.0	24.0	24.0	24.0
D.O. (mg/L)	8.1	8.1	8.1	7.2	8.0	7.7	8.0	7.6
pH	7.37	7.24	7.35	7.24	7.38	7.24	7.36	7.33
Conductivity								
Alkalinity								
Hardness								

15.0 mg/L	Day 0	Day 1		Day 2		Day 3		Day 4
U2723	0 H	Old	New	Old	New	Old	New	Old
Temperature (°C)	24.0	24.0	24.0	24.0	24.0	24.0	24.0	24.0
D.O. (mg/L)	8.2	8.1	8.1	7.2	8.0	7.3	8.1	7.5
pH	7.40	7.26	7.39	7.23	7.41	7.23	7.40	7.39
Conductivity								
Alkalinity								
Hardness								

Definitive Test 2

96 Hour Test Data
Page 3 of 3

25.0 mg/L	Day 0	Day 1		Day 2		Day 3		Day 4
U2723	0 H	Old	New	Old	New	Old	New	Old
Temperature (°C)	24.0	24.0	24.0	24.0	24.0	24.0	24.0	24.0
D.O. (mg/L)	8.2	8.2	8.2	7.1	8.1	7.2	8.0	7.5
pH	7.37	7.27	7.34	7.24	7.32	7.25	7.35	7.34
Conductivity								
Alkalinity								
Hardness								

_____	Day 0	Day 1		Day 2		Day 3		Day 4
	0 H	Old	New	Old	New	Old	New	Old
Temperature (°C)								
D.O. (mg/L)								
pH								
Conductivity								
Alkalinity								
Hardness								

_____	Day 0	Day 1		Day 2		Day 3		Day 4
	0 H	Old	New	Old	New	Old	New	Old
Temperature (°C)								
D.O. (mg/L)								
pH								
Conductivity								
Alkalinity								
Hardness								

96 Hour Test Data

Effluent/Groundwater/Toxicant: 6-Aminonicotinamide Date: 5/22-5/26/00

Analyst: ST

Species: Xenopus laevis Age at Start of Test: 8-11

~~Control~~ 5.5 mg/L (6-AN)	Day 0	Day 1		Day 2		Day 3		Day 4
	0 H	Old	New	Old	New	Old	New	Old
Temperature (°C)	24.0	24.0	24.0	24.0	24.0	24.0	24.0	24.0
D.O. (mg/L)	8.2	8.0	8.1	7.9	8.1	7.8	8.1	7.8
pH	7.10	7.08	7.15	7.11	7.17	7.06	7.13	7.02
Conductivity								
Alkalinity								
Hardness								

2500 mg/L (6-AN)	Day 0	Day 1		Day 2		Day 3		Day 4
	0 H	Old	New	Old	New	Old	New	Old
Temperature (°C)	24.0	24.0	24.0	24.0	24.0	24.0	24.0	24.0
D.O. (mg/L)	8.2	7.9	8.0	7.9	8.0	7.7	8.0	7.6
pH	7.03	7.00	7.08	7.07	7.14	7.03	7.07	6.98
Conductivity								
Alkalinity								
Hardness								

_____	Day 0	Day 1		Day 2		Day 3		Day 4
	0 H	Old	New	Old	New	Old	New	Old
Temperature (°C)								
D.O. (mg/L)								
pH								
Conductivity								
Alkalinity								
Hardness								